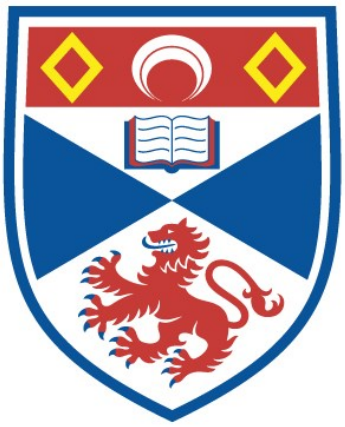
THE ENDOCRINE CONTROL OF REPRODUCTION IN THE RIVER LAMPREY LAMPETRA FLUVIATILIS L.

P. J. Evennett

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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THE ENDOCRINE CONTROL OF REPRODUCTION

IN THE RIVER LAMPREY,

Lampetra fluviatilis L.

by

P. J. Evennett, B.Sc.

Thesis presented for the Degree of Doctor of Philosophy in the Faculty of Science in the University of St. Andrews.

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Declaration.

I hereby declare that the work recorded in this Thesis has been carried out by me except where otherwise stated, and that it is of my own composition. I further declare that it has not been submitted in any previous application for a higher degree.

Research Career.

I received the degree of Bachelor of Science with honours in zoology from the University of Liverpool in July, 1958. The research work recorded in this Thesis was carried out between October 1958 and December 1962. Until September 1960 I held at St. Andrews University research studentship at the Gatty Marine Laboratory, St. Andrews. The work was completed in the Department of Zoology, University of Leeds, while I held the post of research assistant.

Supervisor's Certificate.

I certify that Peter John Evennett has fulfilled the conditions laid down in the regulations for the Degree of Ph.D., under Ordinance No.16 of the University Court of the University of St. Andrews, and that he is accordingly qualified to submit this Thesis for the Degree of Doctor of Philosophy.

Acknowledgments.

I wish to thank my supervisor, Professor J. M. Dodd, for his constant interest and encouragement throughout this work and for criticising the manuscript; also Professor H. G. Callan for advice on cytological problems. Thanks are also due to Bailiff P. J. Gaskins for supplying the lampreys.

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INTRODUCTION.

The major portion of endocrinological research has been devoted to mammals. Among the lower vertebrates most groups have received some attention in recent years in the context of comparative endocrinology, but our knowledge of most of these groups is still meagre.

The reproductive endocrinology of one group of vertebrates, the cyclostomes, appears almost to have escaped attention, and no investigations into the physiological control of reproduction have previously been made. The work reported here was carried out in order to provide some information on the relationship of the pituitary gland to reproduction in the cyclostomes.

It is surprising that the reproductive endocrinology of the cyclostomes has been so long neglected, considering the evolutionary importance of this group. Present day cyclostomes are the only surviving representatives of the most primitive group of vertebrates, the Agnatha. Fossil remains assigned to this group are found in Silurian and

Devonian strata, and are thus thought to have existed between 300 and 400 million years ago. Having branched off the main vertebrate line earlier than any other surviving animal, the cyclostomes might be expected to show some of the characters of the early vertebrates. The work presented here will show that the pituitary gland, functioning in basically the same way as it does in modern mammals, is an extremely ancient organ, possibly as old as any other character of the vertebrate's anatomy or physiology.

MATERIAL AND HUSBANDRY.

The experimental animals were adult specimens of the river lamprey, Lampetra fluviatilis (L.). They were trapped in the River Severn between Gloucester and Tewkesbury, and sent by rail packed in wooden boxes lined with damp sacking. Survival was excellent, usually better than 90%. Eggs of this species hatch in rivers in May and the small ammocoete larvae bury themselves in the mud and feed by filtering small food particles from the respiratory current. The larvae grow to approximately six inches in length, then undergo metamorphosis into adults. The duration of larval life is uncertain, but is possibly three or four years.

After metamorphosis, the young adults swim downstrea to the estuary where they remain for probably two or three years, during which time they feed, possibly parasitically, and grow to ten or twelve inches in length. The anadromous spawning migration begins in late September, and in the following April or May the lampreys select their nests, spawn and the spent animals die. After the animal has re-entered freshwater, feeding ceases and the gut atrophies.

The lampreys were kept in laminated glass fibre tan of approximately 50 gallons capacity in well aerated, constant running tapwater. During preliminary experiments in St. Andrews many lampreys became infected with the fungus

Saprolegnia parasitica, particularly in the region of the operation wound, the infection invariably leading to death within a few days. In later experiments, carried out in Leeds, 25 ml. of a 2% aqueous solution of mercurochrome was added daily to the water of each tank containing experimental animals, and allowed to wash out. Very few lampreys became infected with the fungus during these later experiments, but it is not clear whether this was due to the regular use of mercurochrome or to other factors, such as the shorter rail journey, with less chance of damage to the skin, or the possible presence of a smaller number of fungal spores in the Leeds water.

Although the lampreys maintained in tanks did not spawn, due presumably to the absence of suitable gravel and stones for nest-building, yet they died in April or May, as they do in their natural environment.

TECHNIQUES.

1. SURGICAL TECHNIQUES.

a) Hypophysectomy.

The operation of hypophysectomy performed in the following way is both simple and rapid, and post-operative survival is extremely good, being little, if any, worse than that of unoperated lampreys.

Methanetricaine sulphonate (MS222, Sandoz) was used as an anaesthetic. This substance is superior to the more usua fish anaesthetic, Urethane, in several ways. MS222 is very soluble in water and is potent at high dilutions, a solution of 1 g. in 2.5 litres producing the required degree of anaesthesia in 15 minutes. For a similar effect, urethane of 10% concentration, or stronger, would be required. MS222 appears to have no harmful effects on the lampreys, even when they are immersed in the solution for as long as 1½ hours. It has been suggested (Ball & Cowen, 1959) that urethane may have a carcinogenic effect on human tissues; there is no evidence to suggest a similar effect for MS222.

After anaesthetisation in MS222 for 15 minutes, the lamprey is laid, ventral side up, on the operating board, and fixed to the board by means of a pin passing through the anterior margin of the sucker. (~~Fig.]~~). With a sharp scalpel blade a median longitudinal incision is then made in the skin from just behind the sucker to a point just anterior to the

Fig.1. Hypophysectomy.

Lamprey on operating board with incision made and retractors in position.

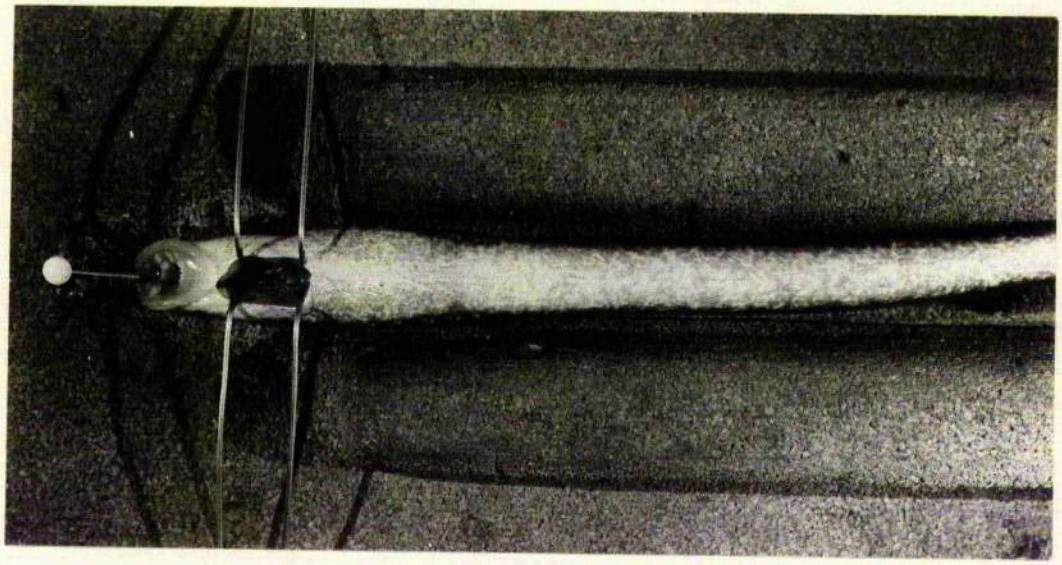
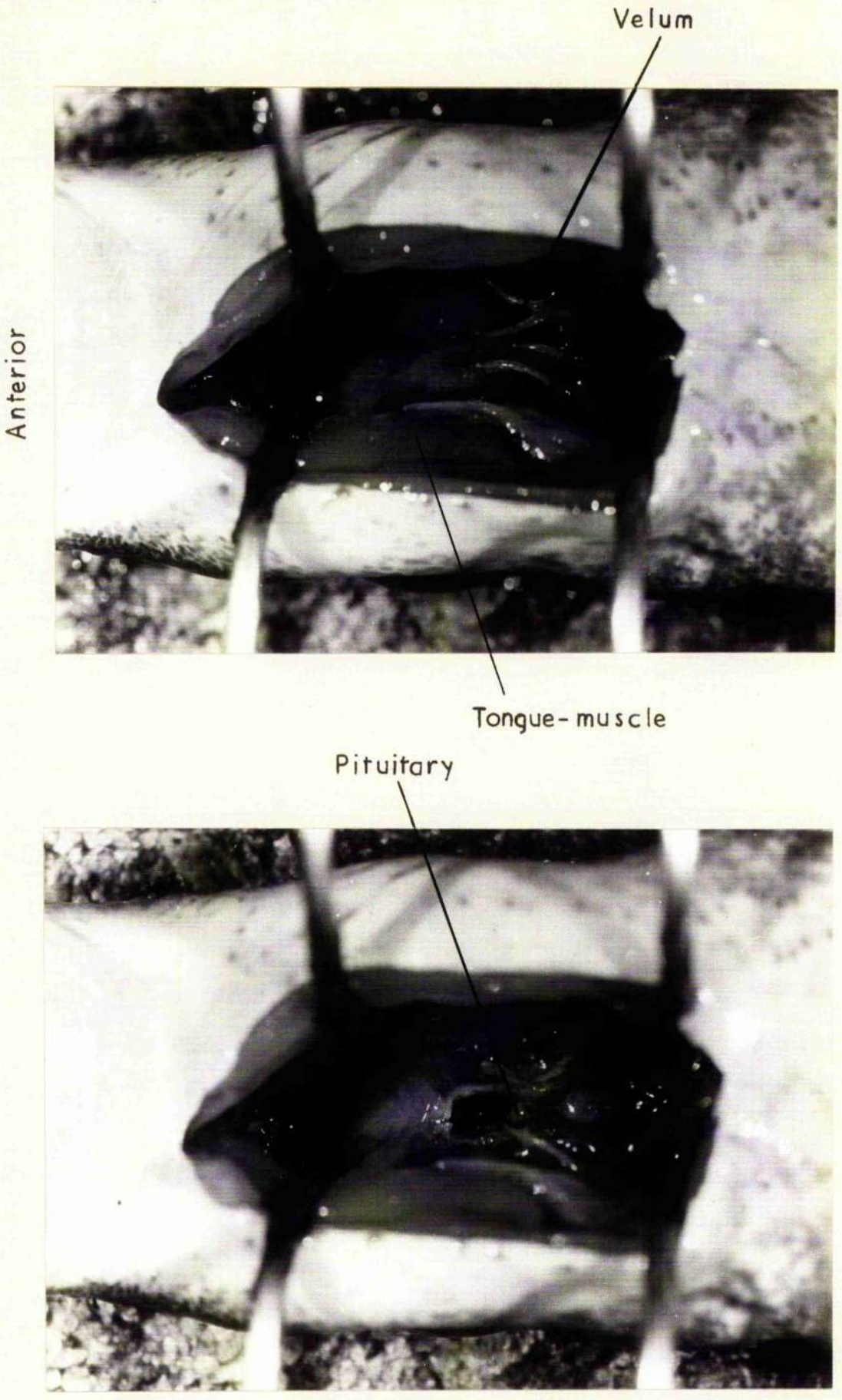


Fig.2. Hypophysectomy.

View into pharynx showing cut edges of tongue muscle, and velum.

Fig.3. Hypophysectomy.

Incision extended into hypophysial canal exposing pituitary.


Velum
Anterior
Tongue-muscle
Pituitary

first pair of gills. This incision is then extended dorsall through the tongue musculature by blunt dissection until it enters the pharynx. As the muscle fibres lie longitudinally they are simply separated, and not unduly damaged, by this incision. It was noted that unimpaired sucker action was important if the animals were to remain in good condition after the operation.

The velum, which marks the mouth of the sub-oesopha tube, is seen when the incision opens into the pharynx. At this stage two stainless-steel wire retractors are inserted into the pharynx through the ventral incision, and the two components of the tongue muscle gently drawn apart (Figs.1, 2 An illuminated, x15 stereoscopic microscope is now swung into position, and a small median cut made in the velum, separatin this into two lateral portions and giving improved access to the pituitary region. The thin dorsal wall of the oesophagu which also forms the floor of the hypophyseal canal, is incised medially just dorsal to the normal position of the velar opening, great care being taken to avoid cutting the carotid arteries lying immediately beneath. This incision is carefully continued anteriorly, up to the point where this thin septum meets the cartilage of the cranium.

The pituitary gland is now visible as an oval, whit patch in the roof of the hypophyseal canal (Fig.3). The anterior and posterior regions of the pituitary - the pro- an

meta-adenohypophysis - are milky-white in appearance, while the centrally placed meso-adenohypophysis is translucent white This fairly clear demarcation into regions makes partial hypophysectomy operations possible, though the precise scope of such operations can only be gauged by subsequent examinatio of the pituitary region by serial sectioning.

In all cases, the pituitary was destroyed by means of an electrically-heated cautery. The wire used for the cautery was a platinum alloy (H-alloy, Johnson, Matthey & Co. Ltd.) of 0.008" diameter (35 SWG), a length of approximately 1" being bent into a very tight U-shape and held in a suitable handle. A current of about 3 amps. at 2 volts (supplied by a transformer controlled by a foot-switch) provides sufficient heat. The cautery wire is heated to bright red heat and the pituitary destroyed, extreme care being taken to avoid contact with the numerous large blood vessels in this region. The resulting cavity in the base of the brain is plugged with a small piece of gelatin sponge to prevent water from the pharyn from entering the ventricles of the brain, the retractors are relaxed, and the edges of the skin sutured with fine gut. Gu was found to be more satisfactory than the nylon sutures used in earlier experiments, as it was less prone to tear the skin.

The duration of the operation is usually between fiv and ten minutes, and respiratory movements begin soon after th lampreys are returned to water. Within about twelve hours th

animals become pale in colour, indicating the absence of the melanophore-stimulating hormone.

Control operations were performed in the way described above, except that the pituitary was left intact.

b) Gonadectomy.

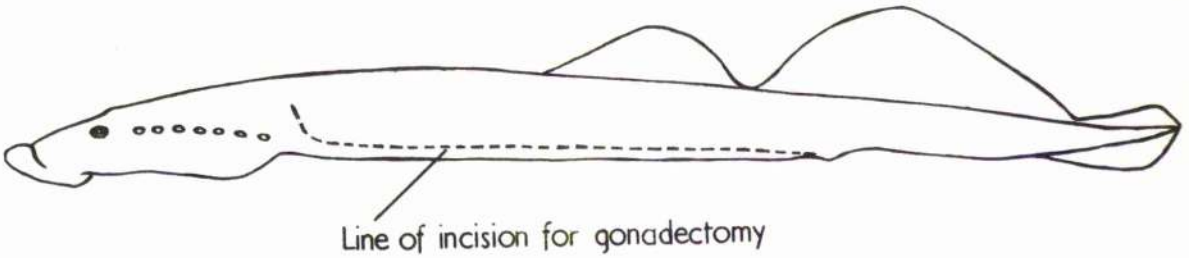
Knowles (1939) described this operation as "impossi due to the size and position of the gonads". It is true tha the position of the gonad, closely applied to the kidneys and the dorsal aorta, and extending anteriorly as far as the live makes this operation an extremely delicate one; however, using the greatest care, and in the absence of fungal infecti at the long operation wound, success can be achieved and the operated animals survive.

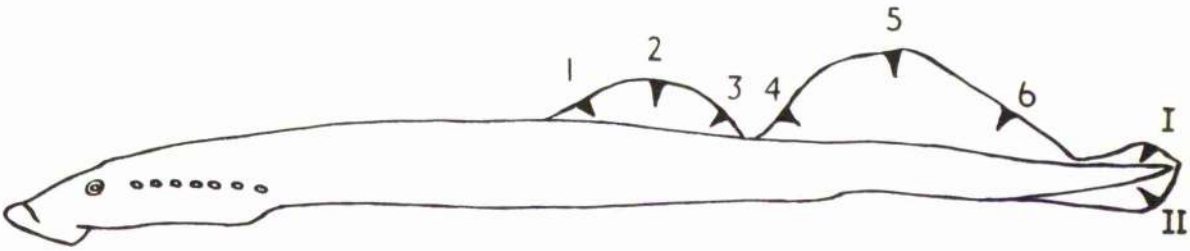
As the duration of the operation was sometimes 30 minutes, the lampreys were more deeply anaesthetised (in MS222) than for hypophysectomy. The anaesthetised animal is laid on its side on a flat operating board and incised with a scalpel in the lateral region of the ventral body wall, approximately half way between the cloaca and the last gill opening. With fine scissors the incision is extended posteriorly to the level of the cloaca, and anteriorly so as to approach the liver. About 1" behind the seventh gill opening, the line of the incision curves dorsally, and terminates just above the posterior tip of the liver (Fig.4).

Fig.4. Gonadectomy.

Line of incision for gonadectomy.

Fig.5. Method of marking showing positions of fin-clippings.


Line of incision for gonadectomy


1
2
3
4
5
6
I
II

The same technique of gonad removal was employed for both male and female lampreys. The gonad is held with blunt forceps, its median attaching mesentery stretched and cut at a point roughly equidistant between the liver and the cloaca, and the gonad separated into halves. Each half is removed separately, the gonad being kept taut by ventral tension with the forceps, and the mesentery carefully severe with a scalpel. Complete removal of the gonad is possible, special attention being paid to its anterior extremity, abov the liver. Greater care is required in the removal of the posterior portion of the gonad, and particular care must be taken to avoid damage to the kidneys, which are in close contact with the gonad.

The wound is stitched with fine gut, using a runni stitch for speed, and before the body cavity is finally clos it is filled with 0.7% NaCl solution. Unless fluid is adde air which becomes trapped in the body cavity causes the animal to float on its return to water.

c) Gonad biopsy.

A small sample piece of gonad was removed from each lamprey prior to hypophysectomy and injection treatment and in some cases samples were removed at various times after operation. A small incision less than ¼" long is sufficient large to allow removal of a small gonad sample, with the aid of fine curved forceps. No suture is required in male anima

to close the biopsy wound, but in some females it was found necessary to insert a single stitch.

d) Injection.

All injected materials, with the exception of Testaform, were administered in solution or suspension in 0.7 NaCl, in volumes of 0.1 ml. or less. Testaform (B.D.H.) is an aqueous suspension of testosterone propionate. Injection were made into the musculature just lateral to the first dors fin, the nature of the tissue in this region allowing very little leakage of the injected fluid.

Extracts of lamprey pituitary were made by grinding fresh or acetone dried glands with saline in an agate mortar for several minutes; the supernatant was then injected witho further treatment.

Pregnant mares' serum gonadotrophin ('Gestyl'), and human chorionic gonadotrophin ('Pregnyl') were obtained from Organon Ltd.

e) Implantation.

Testosterone was administered in the form of 25 mg. pellets (Organon Laboratories Ltd.) implanted into the body cavity through the small gonad biopsy incision. In some cas half-pellets of approximately 12.5 mg. were implanted.

f) Method of marking.

A reliable method if distinguishing individual lampreys is required in an investigation of this nature, thou as far as possible only lampreys treated similarly, or hypophysectomised at the same time, were kept in the same tan

Preliminary experiments on the marking of lampreys by stitching coloured markers to the skin close to the dorsal fins were unsuccessful, as fungus invariably infected the wound, usually resulting in the death of the lamprey. Attem at marking the white ventral surface of the lampreys by tattooing were also unsuccessful due to the nature of the ski Eventually the simplest method of marking - fin clipping - was adopted, with complete success.

Clipping is done with a sharp scalpel, the fin bein laid flat upon a cork-covered board. A narrow, V-shaped portion of the fin is removed, this slight injury never givin rise to any bleeding or causing fungal infections. The cut edges of the fins do not knit together and obliterate the marking during the course of the experiments. The two dorsa fins, and also the dorsal and ventral portions of the caudal fin are used in the marking scheme adopted. Each dorsal fin may be clipped anteriorly, centrally or posteriorly, and thes positions are numbered from 1 to 6 as shown in Fig.5 . On or two dorsal fin clippings are used, if necessary in combina with caudal fin clippings I or II, as shown in the Figure.

This method provides sixty-three different combinations using often only one or two, and never more than three clippings.

2. GRAVIMETRIC TECHNIQUES.

During preliminary investigation it was found that body weight and gonad weight, and therefore the gonosomatic index ($\frac{\text{Gonad wt. x 100}}{\text{Body wt.}}$), were extremely variable among an apparently uniform group of lampreys. Thus in subsequent experiments no record was kept of these weights. One reason for this great variability in gonad weight was that, in the male, as maturity approaches, the testis liquefies, the milt being released into the body cavity. Accurate weighing of the ripe testis was thus impossible.

In the female it was considered that the best gravimetric indication of gonad development was given by the dry weight of a known number of eggs. For convenience, the determinations are made using formalin-fixed ovaries. One hundred eggs are counted and placed in a solid watch glass. The connective tissue of the ovary is so sparse as to have little effect on the final weight, so its presence is disregarded. The watch glasses containing the eggs are heated i an oven to 110°C for 24 hours, and then placed in a desiccator for a further 24 hours. The eggs are weighed to the nearest 0.1 mg.

3. HISTOLOGICAL TECHNIQUES.

a) Fixation.

Similar techniques were employed in treating both the unoperated and experimental lampreys. The lampreys were killed by decapitation and the head dropped immediately into Bouin's fluid (Pantin, 1959). After removal of the gonad the body was also fixed in Bouin's fluid, together with the head, for 48 hours and stored in 70% alcohol.

The gonads were divided into three portions, which were fixed separately. One portion was fixed in Bouin's fluid as above, one in Baker's formaldehyde-calcium (1944) for lipid studies, and the third portion in either Carnoy's fluid (Pantin 1959) (testis) or Smith's formalin-bichromate fixative (Pantin, 1959) (ovary). Gonad samples were fixed in Baker's formaldehy calcium for 48 hours, then transferred to Baker's formaldehyde-calcium-cadmium solution for storage. Testes were fixed in Carnoy's fluid for 12 hours and transferred immediately to 95% alcohol. The Smith's fixative for ovaries was made up fresh before use, and the tissues fixed for 24 hours. After washing for 12 hours in running water they were stored in 4% formaldehyde.

b) Section-cutting.

Early attempts at cutting sections of lamprey pituitary regions embedded in paraffin wax were often unsuccess ful, due to the hardening of the tissues, especially the

cartilage, produced by this technique. The use of polyester wax (Steedman, 1957, 1960) caused much less hardening due to its low melting point (30-35°C) and the fact that tissues can be placed into molten wax from 95% alcohol, thus avoiding total dehydration and the hardening effects of 'clearing agents'. Polyester wax was adopted as embedding medium for testes and ovary sectioning also. The wax was made up to Steedman's 1960 formula:

400 polyethylene glycol distearate	90 g.
cetyl alcohol	10 g.

During part of the work the temperature in the laboratory exceeded 25°C, and at this temperature normal polyester wax was found to be rather too soft for good section-cutting. Following the suggestion of Steedman (1960) 10 g. of Ester Wax 1960 (B.D.H.) was added to 90 g. of the above 90:10 mixtu The resulting wax was found to be considerably harder while retaining the good qualities of pure polyester wax, and its use was continued even in more normal temperatures. Section were cut at between 5 and 10μ and floated on 4% formaldehyde on albumen coated slides. Pituitary glands were cut in the longitudinal plane, and sections close to the mid-line were mounted singly or in pairs to enable several staining techniq to be used on near-sagittal sections.

c) Staining.

Pituitary sections were stained with Heidenhain's Azan technique, as described by Pantin (1959). As a routine histochemical technique, the aldehyde fuchsin (AF) stain according to Gomori (1950) or Gabe (1953) was used. (For formulae and times see Appendix). The periodic acid-Schiff (PAS) technique was used according to the technique of McManus (1948) using Schiff's reagent prepared by the method of Coleman (1938).

Testis sections were stained with Heidenhain's iron alum haematoxylin (Pantin, 1959) or Mayer's haemalum (Gray, 1954) and lightly counterstained with 1% alcoholic light green. Sections of ovaries were stained by the Azan technique.

For the location of lipids in gonad material fixed in Baker's formaldehyde-calcium, sections were cut using a water-soluble embedding medium. After thorough washing in running water, the material was immersed in aqueous solutions of polyethylene glycol 1500 of 25%, 50% and 75% concentration for several hours each. The material was then transferred t the molten wax at 55°C for 4 hours. Sections were cut in the ordinary way at 10μ, and fixed to albumen coated slides by pressing with a cigarette paper beneath the thumb. The slides were then allowed to stand in formaldehyde vapour for 12 hours in order to coagulate the albumen.

Sudan black in saturated solution in 70% alcohol was used to demonstrate the presence of lipids (Pantin, 1959). In order to determine whether cholesterol or its esters were present in the sections, the Schultz reaction was employed, in its improved form after Weber, Philips & Bell (1956).

Fig.6. Sagittal section through the head of a lamprey showing position of pituitary. (X 6).

SUCKER
TONGUE MUSCLE
PHARYNX
VELUM
CANAL
HYPOPHYSEAL
NOTOCHORD
BRAIN
PITUITARY
INFUN

MORPHOLOGY AND HISTOLOGY OF STRUCTURES IMPLICATED IN REPRODUCTION.

1. THE PITUITARY GLAND.

The pituitary gland of the lamprey takes the form of a flattened plate of cells situated in the floor of the third ventricle of the brain. The pituitary consists of a neural and a glandular portion, the latter being subdivided transversely into three regions. The glandular adenohypophysis consists, according to the terminology of Pickford & Atz (1957 at its anterior end of the pro-adenohypophysis, behind which is found the meso-adenohypophysis, Caudal to the meso-adenohypophysis lies the meta-adenohypophysis, beneath the neurohypophysis, which is continuous with the layer of ependymal cells that separates the pro- and meso-adenohypophys from the third ventricle. The ventral surface of the pituitary is in contact with the thin connective tissue and mucous membrane roof of the nasohypophyseal canal (Figs.6, 7).

a) Review of Literature.

i) Staining methods for pituitary cells.

Working with traditional staining methods, for examp the trichrome Mallory's triple stain (Mallory, 1900) and its modification, Heidenhain's Azan (Heidenhain, 1915), three cell types have been recognised in the adenohypophysis of most vertebrates; these are the acidophils, basophils and

chromophobes, taking their names from the staining reaction of the granules present within the cytoplasm.

In some species acidophils of two kinds have been distinguished: in the Azan technique the granules of certain acidophils stain with orgnge G, while those of other cells stain with the azocarmine. Among the lower vertebrates this has been found in fishes (Teleostei, several spp., Scruggs, 19 and amphibians (Triturus viridescens, Copeland, 1943; Necturus and Rana catesbiana, Green, 1951). In mammals the acidophils are thought to be the source of the somatotrophic hormone and prolactin.

By the use of the periodic acid-Schiff (PAS) reactio of McManus (1946) and similar techniques, in combination with other stains, four types of basophils have been identified in the human adenohypophysis. In most other vertebrates two or three types of basophils have been distinguished, though in many cases the staining techniques have been developed for use with mammalian glands and their suitability for use with lower vertebrate material is often questionable.

It is generally agreed that all cells now described as basophils contain granules which give a positive PAS reaction, and that this reaction is due to the presence of soluble glycoproteins (Purves, 1961). In most groups of vertebrates these PAS-positive cells have been shown to secrete either thyroid stimulating hormone or the gonadotrophi hormones which are known to be glycoproteins (Li, 1949).

The aldehyde fuchsin technique (AF) in its several forms (Gomori, 1950; Halmi, 1952; Gabe, 1953) stains, in most vertebrates, some but not all of the PAS-positive basoph In the rat Halmi (1950, 1952) and Purves & Griesbach (1951a, b have concluded that the basophils which are selectively stain by the AF-method are the thyrotrophs, while the gonadotrophs are AF-negative. The AF technique has been used in teleosts to differentiate between thyrotrophs and gonadotrophs by Barrington & Matty (1955) using Phoxinus phoxinus, where only the cells they regarded as thyrotrophs gave a positive AF reaction. On the other hand, Atz (1953), working with Astyanax mexicanus, and Barr (1962), working with Pleuronectes platessa, found that all the adenohypophyseal cells which stained with PAS also gave a positive AF reaction.

In the cyclostome Myxine glutinosa Matty (1960) claimed to have identified "at least two different types of basophils" using Heidenhain's Azan or Mallory's triple stain, though he gave no details of the differences in staining of th cells. PAS-positive material was found within these basophil and the author reported that "some cells also stained after applying the Gomori aldehyde-fuchsin technique". It is not clear from this statement whether all, or only some, of the PAS-positive cells reacted with AF.

At present the chemistry of the AF reaction is not understood, and solutions prepared according to different

formulae, or from different batches of basic fuchsin, may give different results (Purves, 1961). Thus the results must be interpreted with caution.

It has also been possible in some cases to distingui between two types of gonadotrophs. In mammalian studies, differentiation between thyrotrophs and two types of gonadotrophs has been carried out by Wilson & Ezrin's (1954) technique using PAS in conjunction with orange G and methyl blue. In the rat these authors found that the thyrotrophs stained with PAS alone, and the gonadotrophs stained with PAS together with either orange G or methyl blue. Barr (1962), working with Pleuronectes platessa, has found that all the basophilic cells of the pituitary remain PAS-red after the Wilson & Ezrin procedure.

ii) The lamprey pituitary gland.

The embryonic development and gross anatomy of the lamprey pituitary gland have been studied by many authors (Herring, 1913; Stendell, 1914; Woerdeman, 1914; de Beer, 1923; Tilney, 1937; Leach, 1951; Herlant, 1954), but until recently its cytology has not been studied in detail.

Roth (1957) in his description of the 'pars distalis' (pro- and meso-adenohypophysis) of the sea lamprey (Petromyzon marinus) recognised five cell types:

a) Large cells, differentiating just before metamorphosis, PAS and AF positive, found in

pro-adenohypophysis and posterior meso-adenohypophysis.

b) Cells containing large Bodian-protargol positive granules and small PAS-positive granules, found in pro- and meso-adenohypophysi of spawning adults.

c) Acidophils, differentiating just before metamorphosis in the dorsal region of the meso-adenohypophysis.

d) Large chromophobes in meso-adenohypophysis.

e) Small chromophobes in pro- and meso-adenhypophysis.

No attempt was made to attribute function to the various typ of cell.

The most detailed study of the lamprey pituitary i that of van de Kamer & Schreurs (1959) working with the brook lamprey (Lampetra planeri). These workers assigned functio to the cell types found in each region of the gland, though their opinions are based solely on histological evidence.

In the pro-adenohypophysis, van de Kamer & Schreurs described only one cell type and this was basophilic and PAS-positive. From examination of larval and metamorphosing lampreys they found that the quantity of secretion stored in the cytoplasm of these cells increased up to the time of metamorphosis, and declined thereafter, the cells shrinking and showing "few signs of activity" after spawning.

Dilatation of the capillaries of the pro-adenohypophysis was also maximal during metamorphosis. The brook lamprey spawns in the spring following its metamorphosis in the autumn, and the gonads actively develop during metamorphosis. From this evidence, and from the fact that, at the time when growth of the oocytes begins in female larvae, these cells of the pro-adenohypophysis are the only basophils stainable within the pituitary, they conclude that the pro-adenohypophys is the source of the gonadotrophic hormone.

Two cell types were found within the meso-adeno-hypophysis. Cells of the first type were chromophobes whose nuclei contained small basophilic granules. The authors found that the granules disappeared during metamorphos the cell diameter increased to twice its pre-metamorphic value and the blood supply to the meso-adenohypophysis increased. After metamorphosis these cells reduced to their normal diameter and the basophilia of the nuclear granules returned. Van de Kamer & Schreurs interpreted the changes in these cells during metamorphosis as denoting great activity, and conclude that these chromophobes may be the source of the somatotrophic hormone. The other cells found in the meso-adenohypophysis were less numerous than the former. These were small, irregularly-shaped basophils with triangular nuclei. The granules of these cells stained with both PAS and AF, the density of staining increasing towards metamorphosis. In

the pre-spawning adult they were surrounded by droplets of basophilic secretion exuded from the cells, the cells themselves having reduced in volume with the production of the secretion. After spawning, these cells shrank and assumed a circular contour, and lost their affinity for basic dyes, PAS and AF. Because of the strong basophilia of the cells during the period of metamorphosis, the authors concluded that the thyrotrophic hormone is secreted by these small cells of the meso-adenohypophysis.

van de Kamer & Schreurs concluded that species differences accounted for the absence of acidophils in the pro- and meso-adenohypophysis of the brook lamprey, while acidophils were found by Roth (1957) in the dorsal region of the meso-adenohypophysis of the sea lamprey.

In the meta-adenohypophysis, van de Kamer & Schreurs found only one cell type: elongated cells lying perpendicular to the infundibular wall. At metamorphosis a granular secretion appeared at the dorsal ends of the cells, the secretion being PAS-positive and also staining with azocarmine. The secretion was released into the capillaries lying between the meta-adenohypophysis and the neurohypophysis. After metamorphosis the secretion decreased in amount progressively, the gland appearing exhausted after spawning had taken place. The appearance of the secretion at the beginning of metamorpho coincided with the establishment of the colour pattern of the skin; from this evidence, and the experimental results of

Young (1935), van de Kamer & Schreurs concluded that the cells of the meta-adenohypophysis produce a melanophore-dispersing hormone.

The neurohypophysis was described as consisting of fibres along which neurosecretory material could be seen, staining deeply with Gomori's chrome haematoxylin phloxin method. The quantity of neurosecretion present decreased at metamorphosis, the fibres becoming nearly totally deprived of the material after spawning, with only small granules remaining. van de Kamer & Schreurs assumed that the neurohypophysis contains the water balance principle, the existence of which in the lamprey was shown by Lanzing (1954) and Sawyer (1955).

van de Kamer & Schreurs mentioned the occurrence of a cyst within the meso-adenohypophysis of an exceptionally large lamprey. They concluded that the abnormally large size of the lamprey was due either to overproduction of somatotrophic hormone by the cells of the cyst, assuming these cells to be secretory chromophobes, or to decreased thyrotrophic hormone production due to the cyst's being enlarged at the expense of the basophils of the meso-adeno-hypophysis. Similar cysts have been recorded in Lampetra fluviatilis by Lanzing (1959) where they occurred in all three regions of the adenohypophysis. In Lanzing's opinion these cysts were lined with cells derived from the epithelium of the nasopharyngeal canal, and in most cases he was able to

find strands connecting this epithelium with the lining of the cysts. Lanzing found that the cysts increased both in volume and number with time, especially in the pro-adenohypoph where he regarded as "remarkable" the fact that "many surround gonadotrophs are hampered in their secretory activity".

In the pro-adenohypophysis Lanzing found two cell types: large basophils, PAS and AF-positive, and staining with aniline blue, similar to those described by van de Kamer & Schreurs for the brook lamprey (above) and also smaller chromophobes, cells which Lanzing considered may "possibly become gonadotrophs, since the gonadotrophs increase in number with time, mitotic divisions, however, being rare". Like van de Kamer & Schreurs, Lanzing regarded the basophils of the pro-adenohypophysis as the gonadotrophs, and found that these cells increased in number and size towards the end of the migratory period.

In the meso-adenohypophysis Lanzing found small basophils reacting to PAS (though less intensely than those of the pro-adenohypophysis) and strongly to AF. These basophils were irregularly shaped and smaller than those of the pro-adenohypophysis, some appearing to be actively secreting throughout the migratory period and others having decreased in size, possessing pycnotic nuclei. Lanzing suggested that these basophils were the thyrotrophs. Most of the cells in the meso-adenohypophysis were found to be chromophobes, some of which appeared to be involved in a

secretory cycle, being enlarged and surrounded by vacuoles. No acidophils were found in any region of the pituitary, Lanzing suggesting that the chromophobes of the meso-adenohypophysis might be functionally related to the acidophils, and that secretion of somatotrophin by these cells occurred during the growth phase of the lampreys, when at sea.

Lanzing found that the meta-adenohypophysis was composed of only one cell type, the cytoplasm of which was either faintly acidophilic or full of basophilic and AF-positive granules. These granules were frequently present at the apical ends of the cells, close to the capillaries whic separate the meta-adenohypophysis from the neurohypophysis. The author regarded the constant activity of the meta-adenohypophysis throughout the migratory period as consistent with its production of melanophore-dispersing hormone.

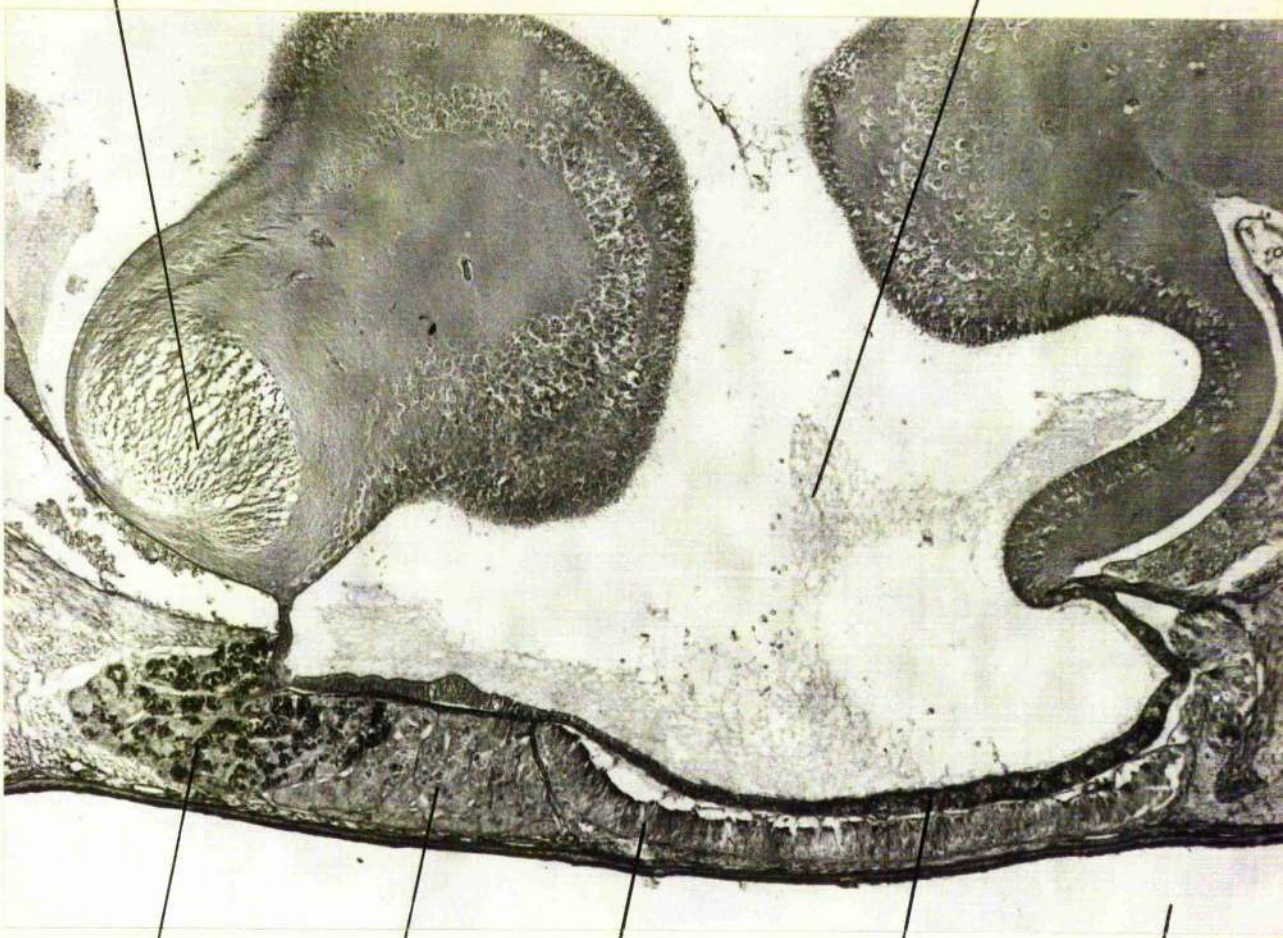
The neurohypophysis was found by Lanzing to consist of a thin layer of fibres extending from the hypothalamus, containing neurosecretory material reacting positively to the AF method.

b) Results of histological examination.

Pituitary glands were examined from a large number of lampreys fixed at intervals between October and April. Occasional sections were stained with Heidenhain's Azan, but routine staining was by the Periodic acid-Schiff (PAS) or the Aldehyde fuchsin (AF, after Gomori and Gabe) techniques.

Fig.7. Longitudinal section through pituitary region of lamprey. (X 70).

Fig.8. Longitudinal section showing pro- and meso-adenohypophysis, and distribution of basophils. (X 100).


Optic chiasma
Third ventricle
Pro-
Meso-
Meta-
-adenohypophysis
Neurohypophysis
Hypophyseal canal

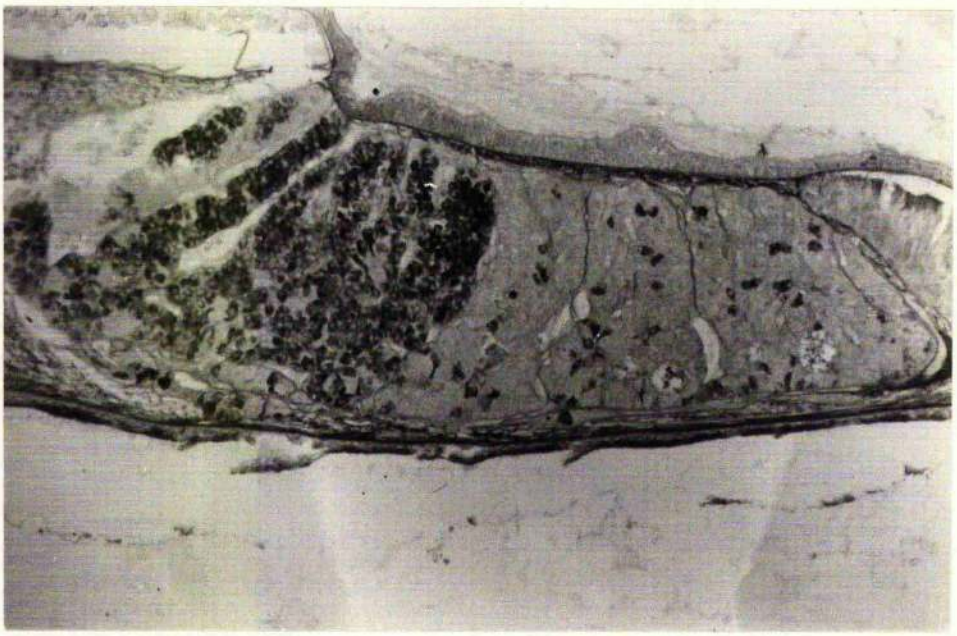
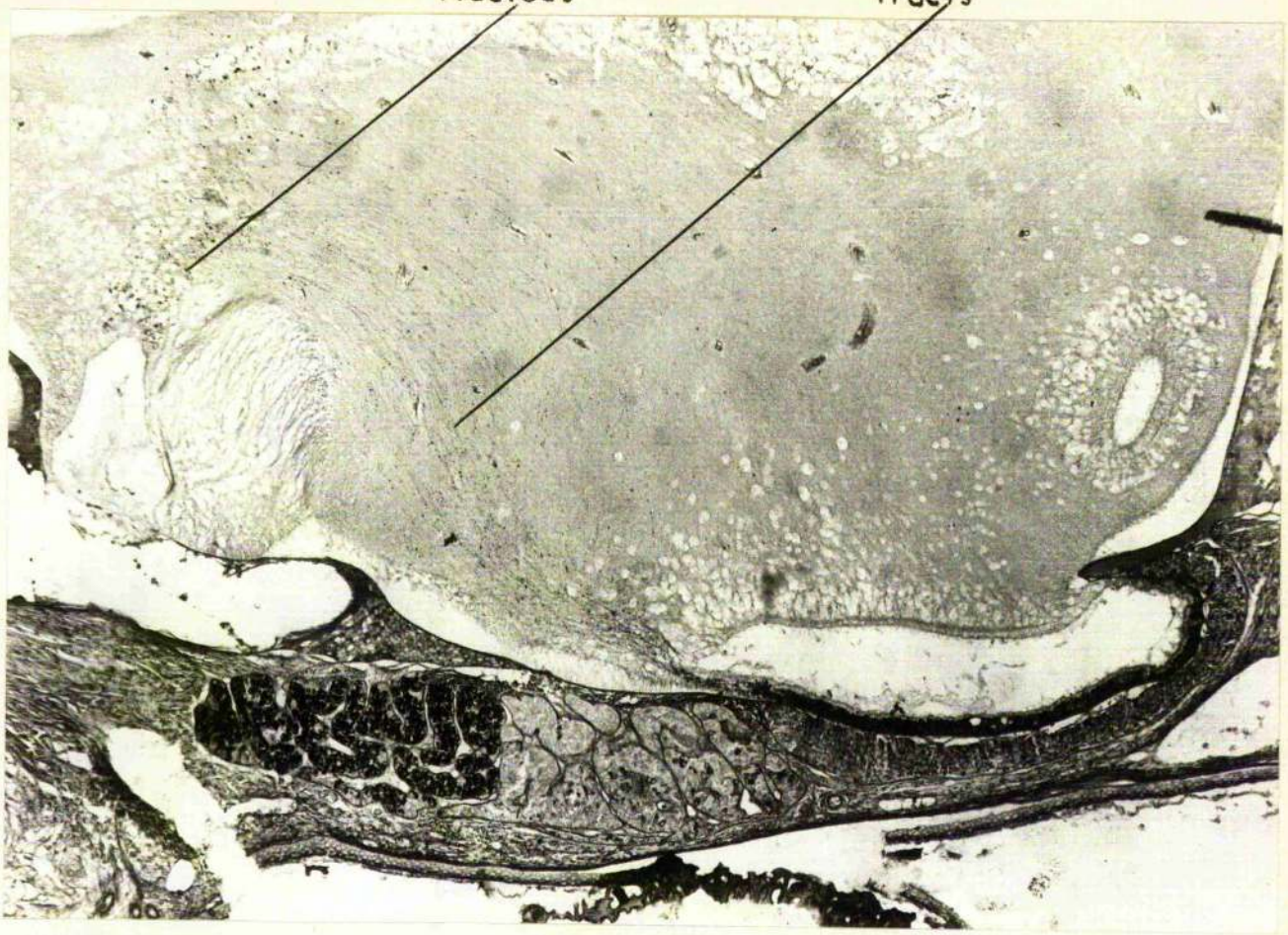
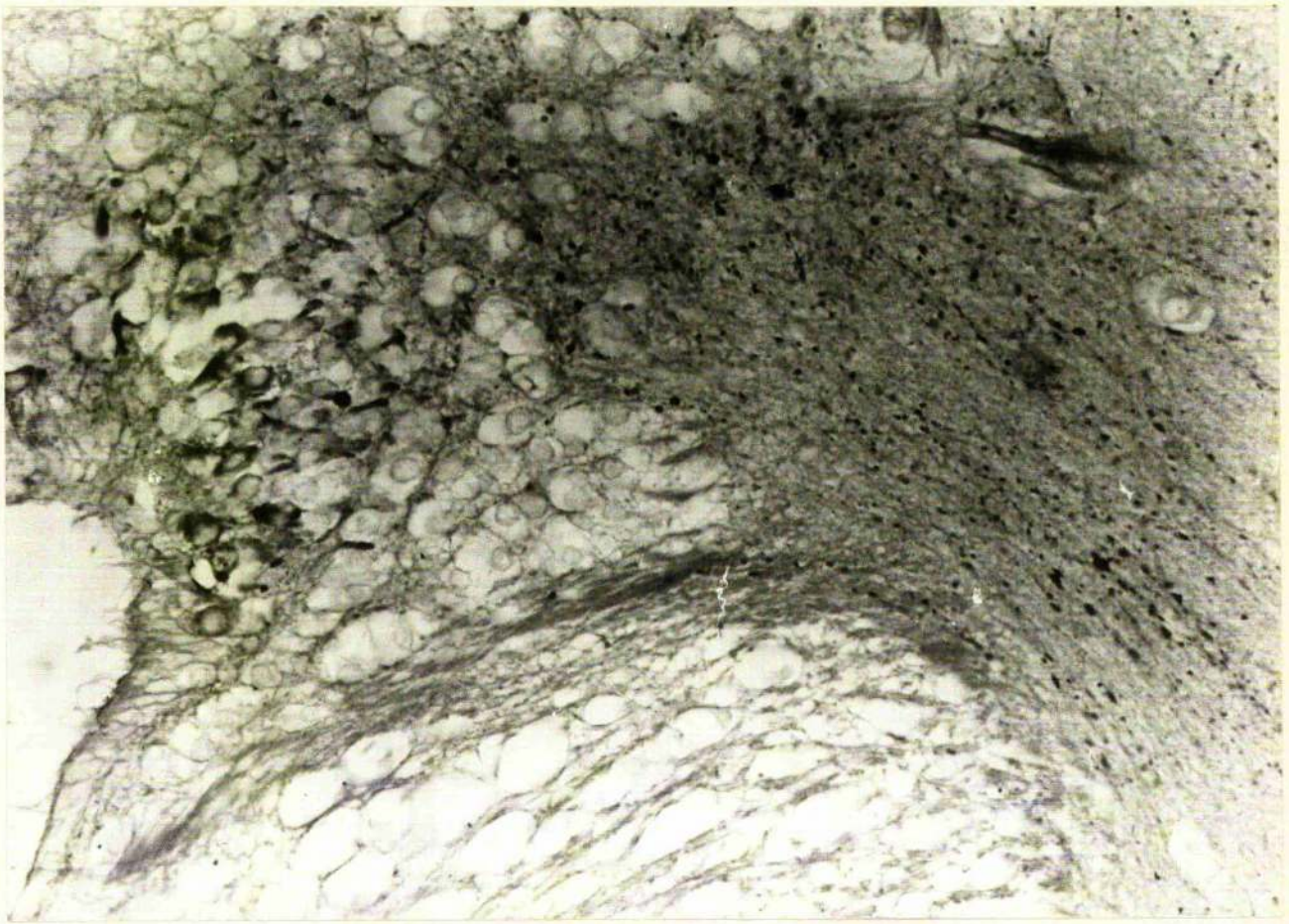


Fig.9. Longitudinal section through pituitary region, stained with aldehyde fuchsin, showing neurosecretory material in the supra-optic nucleus and in tracts leading to the neurohypophysis. (X 70).

Fig.10. High power view of supra-optic nucleus of above. (X 400).


Nucleus
Tracts



Aniline blue basophils were found in the Azan stained sections in large numbers in the pro-adenohypophysis, and in smaller numbers in the meso-adenohypophysis. Staining of contiguous sections with the PAS and AF techniques showed a distribution of positively-reacting cells exactly similar to that of the aniline blue basophils (Fig. 8).

It was concluded that the basophils of both the pro- and the meso-adenohypophysis were also PAS and AF-positive.

No acidophils were seen in the Azan stained sections, or in sections treated with stains containing acid fuchsin. Many chromophobes were seen, these being the only cells other than the basophils found in the pro- and meso-adenohypophysis. In Azan stained sections all the cells of the meta-adenohypophy appeared to be chromophobic, but in some animals the tips of the cells bordering the neurohypophysis in the anterior part of the meta-adenohypophysis were faintly PAS and AF-positive.

The neurosecretory material within the neurohypophysi stained slightly with the azocarmine component of Azan stain, did not react to PAS, but was strongly AF-positive. In the AF-stained sections clearly defined tracts could be seen, running between the pre-optic neurosecretory nucleus and the neurohypophysis (Figs. 9, 10).

Seasonal variation in staining.

In the pituitary glands of lampreys taken at intervals between the upstream migration and spawning, no variation in staining was seen in the pro- or meta-adeno-

hypophysis, or in the neurohypophysis. In the meso-adenohypophysis, however, a considerable variation in the number of AF and PAS-positive cells was recorded throughout the season.(Figs.11-19).

In order to give a quantitative indication of the variation in these cells, cell counts were made. In all cases median longitudinal sections were chosen. The meso-adenohypophysis was photographed and the AF-positive cells counted on the photograph. Two independent counts of each section were made, the results agreeing very well; the mean of the two counts is quoted here.

In a sagittal section through the meso-adenohypophysis of early migrants, in October, between 50 and 60 AF-positive cells were counted. The number of these cells fell to approximately 10 in early February, and then increased again, approaching the original number in April. The results from 21 lampreys are shown in the graph (Fig.20).

The presence of cysts in one or more regions of the adenohypophysis has been reported by van de Kamer & Schreurs (1959) for Lampetra planeri and by Lanzing (1959) for L.fluviatilis.

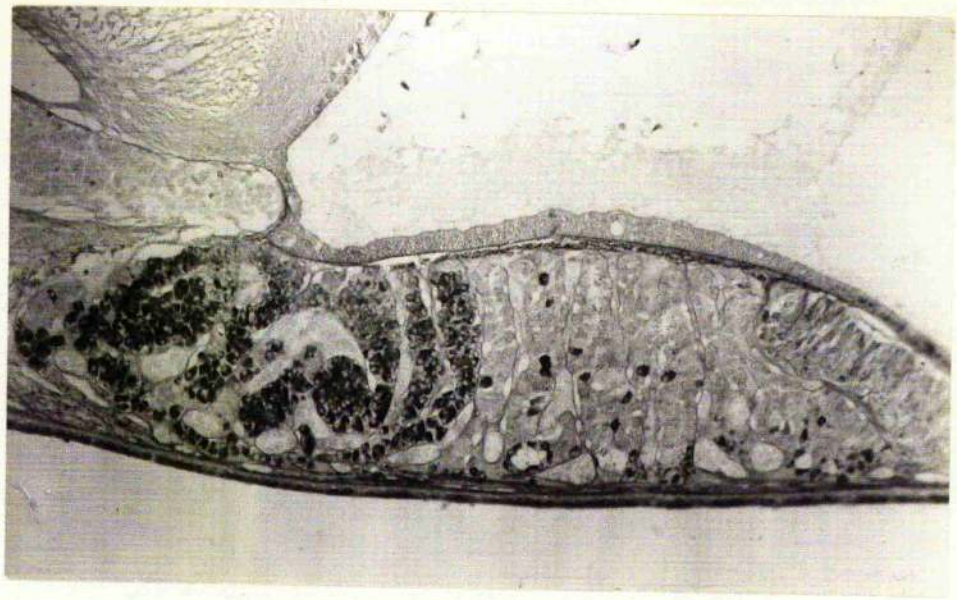
Cysts were also found in this study, in all three regions of the adenohypophysis of approximately half the lampreys examined. There was no obvious connection between the occurrence or size of the cysts and the time of year.

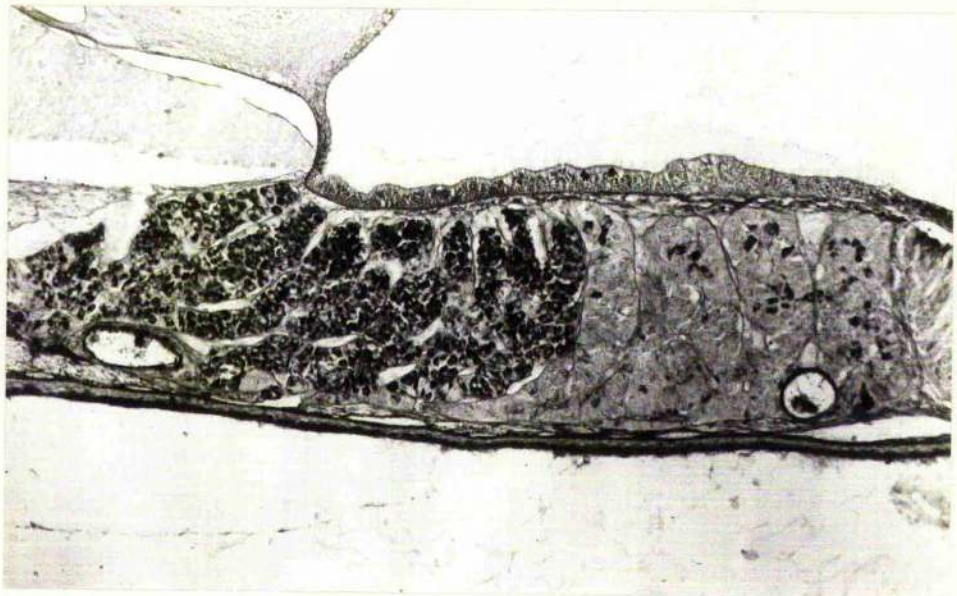
Figs.11-19. Longitudinal sections showing pro- and meso-adenohypophysis, stained with aldehyde fuchsin, to illustrate variation in number of AF-positive cells in meso-adenohypophysis throughout the spawning migration. (X 100).

Fig.11. October 14.

Fig.12. December 29.

Fig.13. January 17.





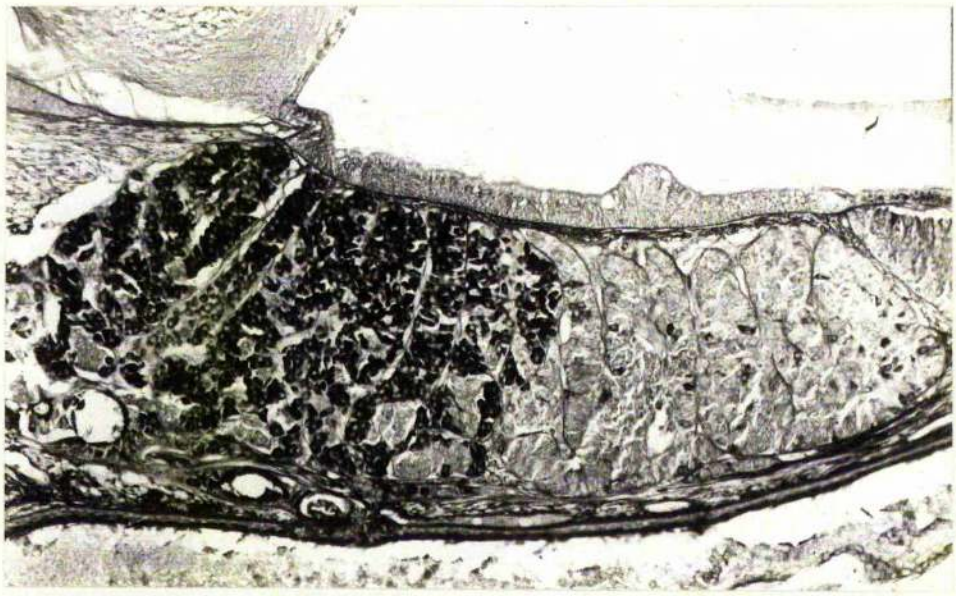
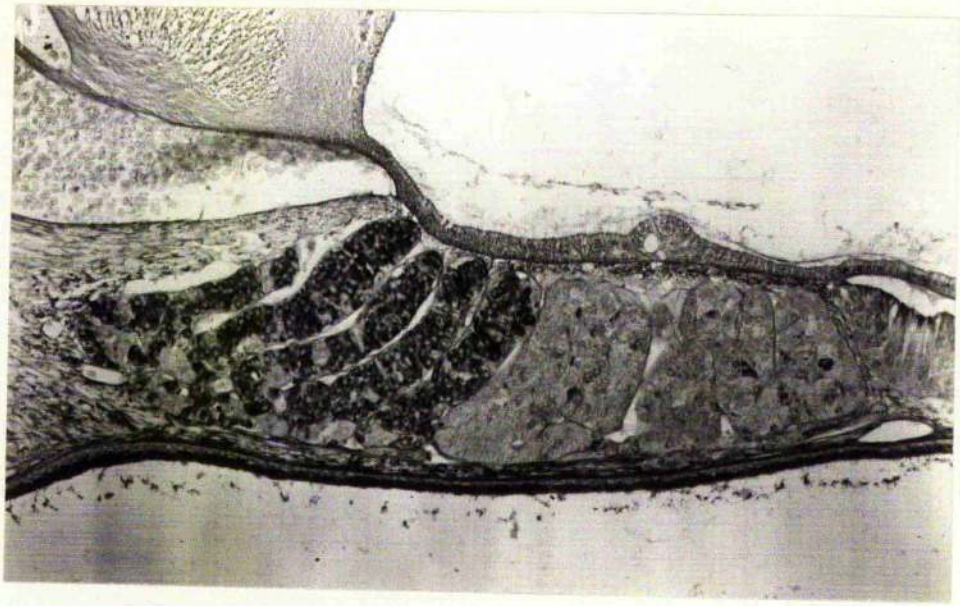
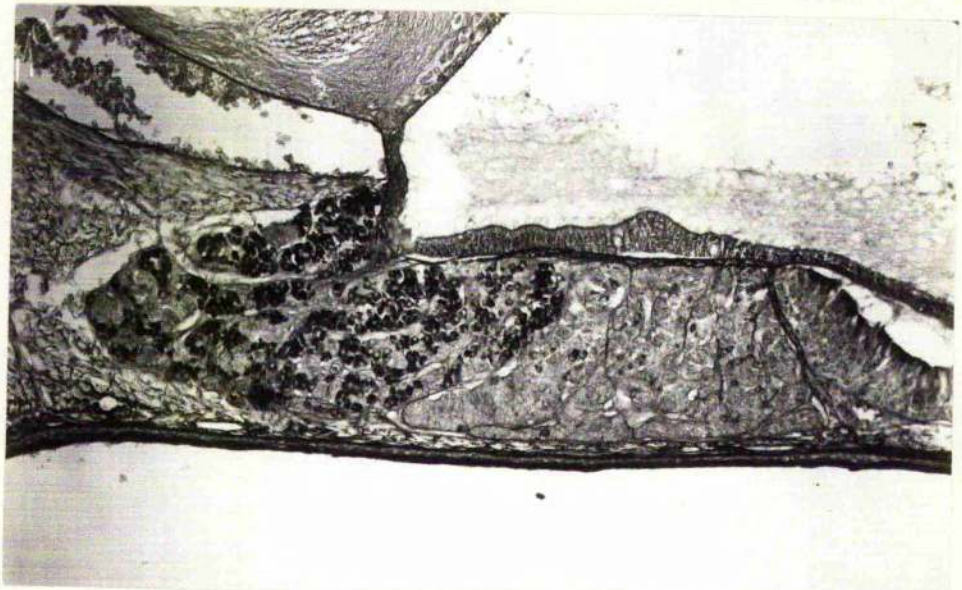


Fig.14. January 23.

Fig.15. February 9.

Fig.16. March 1.





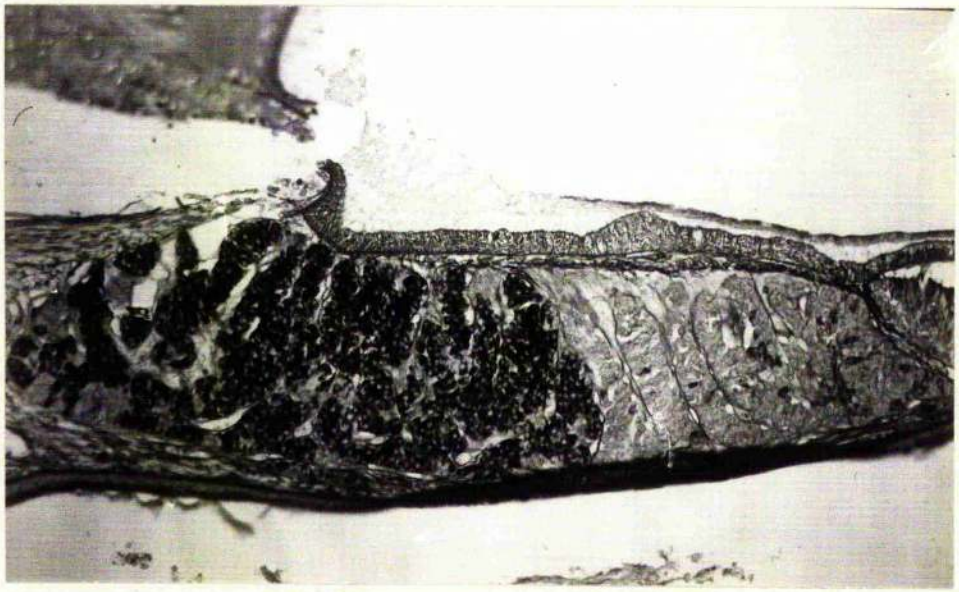
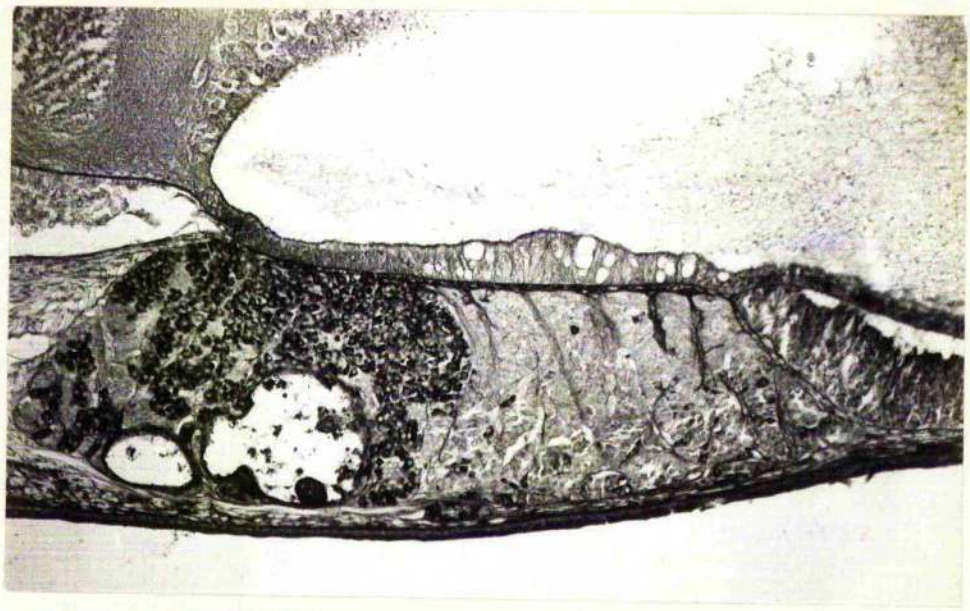
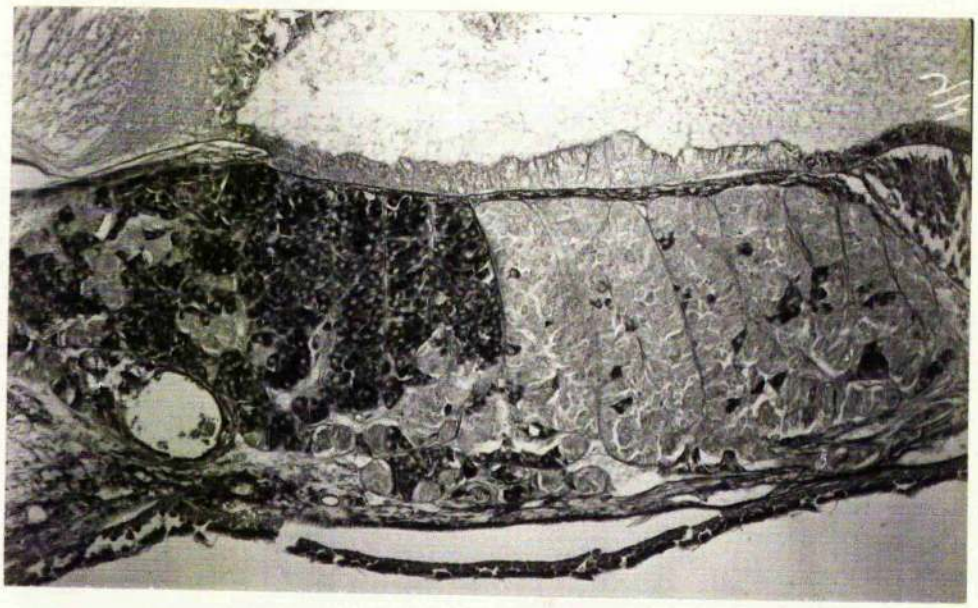


Fig.17. March 12.

Fig.18. March 23.

Fig.19. April 13.





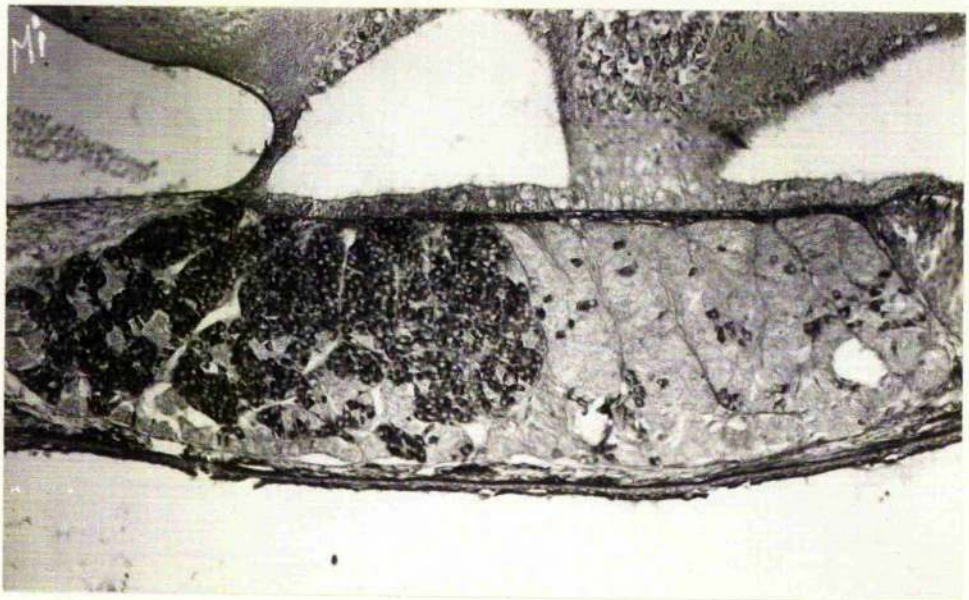
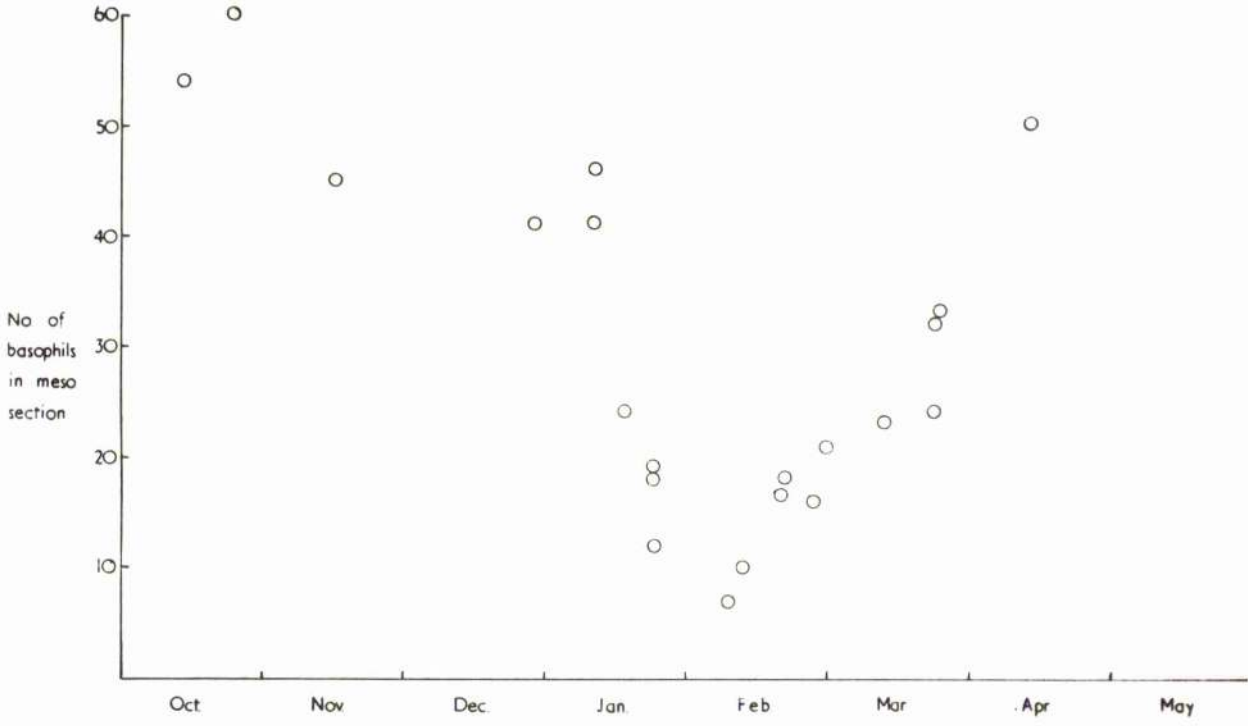


Fig.20. Graph showing the number of cells reacting with aldehyde fuchsin, seen in one sagittal section of the meso-adenohypophysis, taken from lampreys during the spawning migration. (Figures below).

Date	No. of AF-positive cells
Oct.14	48
Oct.24	50
Nov.16	43
Dec.29	41
Jan.10	35
Jan.10	40
Jan.17	23
Jan.23	11
Jan.23	15
Jan.23	15
Feb. 9	5

Date	No. of AF-positive cells
Feb.12	8
Feb.20	11
Feb.21	16
Feb.27	16
Mar. 1	21
Mar.12	23
Mar.23	32
Mar.23	24
Mar.24	32
Apr.13	42

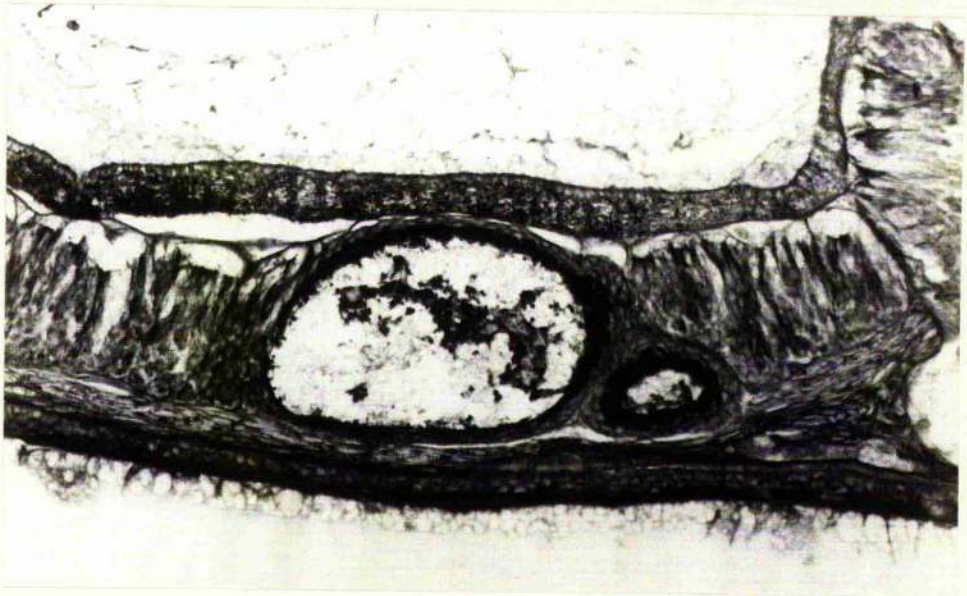

No of basophils in meso section
60
50
40
30
20
10
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May

CYSTS IN ADENOHYPOPHYSIS.

Fig.21. Cyst in pro-adenohypophysis. (X 120).

Fig.22. Cyst in meso-adenohypophysis. (X 120).

Fig.23. Cyst in meta-adenohypophysis. (X 120).



The appearance of the cells lining the cysts was in agreement with Lanzing's observation that these structures are derived from the epithelium of the nasohypophyseal canal (Figs.21, 22, 23).

The Gonads.

In cyclostomes the gonad is a median, unpaired structure extending the whole length of the body cavity from the liver to the cloaca and occupying, during adult life, the greater part of the body cavity. There are no genital ducts, the genital products being shed into the body cavity, whence they escape through the urinary duct via pores which become patent at the time of maturity (Knowles, 1939).

2. THE TESTIS.

a) Review of literature.

There is very little information available on spermatogenesis in the lamprey. Okkelberg (1921) has described the anatomy of the testis in the brook lamprey, but his cytological description of the testis refers only to larvae up to the stage of sex differentiation. The cytology of the germ cells during spermatogenesis has been described only by Chubareva (1958) and by Lanzing (1959).

Chubareva (1958) described the cytological changes occurring in the testis of adult Lampetra fluviatilis (erroneously described as the "river minnow" in the translated

paper) during its spawning migration in the USSR. Adult lampreys caught between September and February were described as the "autumn race" and those caught during May as the "spring race"; spawning lampreys were found in June. These dates suggest that in the Russian river from which these animals were obtained, the lampreys begin their upstream migration at approximately the same time as the lampreys in the English Severn, but that spermatogenesis occurs at a slower rate, the Russian lampreys spawning in June and the English ones in April.

In the lampreys caught between September and Februa Chubareva found only primary spermatocytes in the testes, in the stages of leptotene, diplotene and pachytene. No maturation divisions were found. The nuclei of the primary spermatocytes were larger than those of spermatogonia which were found in ammocoetes, and the former cells had an almost indistinguishable investment of cytoplasm. Within the nucle of early primary spermatocytes the chromosomes were in the form of thin and thick threads, which later shortened and became distributed around the edge of the nucleus during diakinesis. The spindles of the first meiotic division were found to be barrel-shaped, and those of the second division were much elongated. Secondary spermatocytes were smaller than, and spermatids a quarter the size of primary spermatocytes. In the lampreys caught on May 20th, all stages of

spermatogenesis between primary spermatocytes and spermatids were found, and in all the lampreys caught on May 30th the germ cells were in various stages of spermateleosis. On June 16th only ripe lampreys containing solely mature spermatozoa were found.

In his study of the migrating river lamprey (Lampet fluviatilis), Lanzing (1959) has described the changes occurri in the testis. Lanzing's animals were obtained from the Meu in Holland, first becoming available during July and August, between one and two months earlier than our supply from the Severn; spawning occurred during April. In the testes of the early migrants, caught in July, August and September, Lanzing found that the germ-cells were at the spermatogonial stage, numerous mitotic spindles indicating that these cells were still multiplying. One or two nucleoli were occasionall seen within the nuclei of the spermatogonia. By October, the germ cells had reached the stage of primary spermatocytes, many being in the 'synizesis' (leptotene) stage of meiotic prophase with the chromosomes accumulated ~~(leptotene)~~ along one side of the nucleus. Prophase continued throughout November, and metaphases of the first meiotic division were seen during December. Lanzing found that secondary spermatocytes and spermatids were present in January, and that spermatozoa were present im most testis lobules by February. During March little change occurred in the appearance of the testis, though

breakdown of the lobule walls was beginning. By April the testes had completely liquefied and the sperm were free within the body cavity.

Comparison of the accounts of spermatogenesis of Chubareva and Lanzing, both using Lampetra fluviatilis, shows considerable differences of timing. Lampreys entered the Russian river during September and sperm were not found until the end of May, spawning occurring in June; in the Meuse lampreys were available from July and sperm were present in late January, the lampreys spawning in April. These differe are undoubtedly due to the different conditions in the two rivers. Chubareva found that primary spermatocytes were present in the testes when the lampreys first entered the river, and she does not report the presence of any mitotic (or other) spindles which would suggest that this was not so. On the other hand, the many mitotic spindles found by Lanzing in September and earlier prove conclusively the presence of spermatogonia in his material. It appears, then, that the development of the lamprey testis is very dependent upon local external conditions.

b) Results of histological examination.

The testis is a large organ suspended by the median mesorchium within the body cavity and consisting of numerous lamellae situated one behind the other. The whole of the testis is divided into near-spherical ampullae, each bounded

by a thin layer of connective tissues, and containing a solid mass of germ cells. In section, the ampullae are polygonal, and at the angles formed by the intersection of the walls are found cells which display a positive reaction for lipids throughout the period of the spawning migration. The possibility that these 'interstitial' cells are homologous with the Leydig cells of mammals is discussed later.

The division of the germ cells is almost synchronou throughout all the ampullae of an individual testis, and within the population there is a spread of no more than four weeks. All spermatogonia undergo maturation divisions, and at the time of spawning all the germ cells of the testis have been converted into spermatozoa. This is no doubt related to the fact that lampreys spawn only once in their life histo

I) Spermatogenesis.

In late September, when the lampreys first become available at Tewkesbury, the testes, without exception, contain only spermatogonia, these cells still multiplying by mitosis.

Spermatogonia.

Spermatogonia are small cells with sparse cytoplasm and a nucleus of between 5 and 6μ in diameter (Fig. 24). The chromosomes generally form a loose reticulum, and occasionally a prominent 'nucleolus' is visible. Mitotic metaphases are observed throughout October and early November,

the spindles being 5-6μ in length, with an equatorial diameter of approximately 5μ.

Primary spermatocytes.

During November the spermatogonia transform into primary spermatocytes and enter the prophase of the first meiotic division. The chromosomes are then clumped to one side of the nucleus in the typical synizesis or 'bouquet' stage of leptotene (Fig.25), the nucleus being approximately 7μ in diameter. This indicates a slight increase in nuclear size during the transformation from spermatogonia to primary spermatocytes.

Leptotene continues throughout December and January, and it is not until mid-February that the stage of diakinesis is reached, the chromosomes being arranged around the nuclear membrane (Fig.26). The diameter of the nucleus remains constant at 7μ. Diakinesis is followed, at the end of Februa by the appearance of meiotic first metaphase plates. First metaphase spindles can be distinguished from those of the second metaphase by their occurrence within clumps of primary spermatocytes. They are rather barrel-shaped, approximately 7μ in length and 5μ in diameter, and the chromosomes are so tightly clumped as to be individually indistinguishable (Figs. 2 28, 2

Secondary spermatocytes.

Secondary spermatocytes, formed by the first meiotic division, have small nuclei, approximately 3μ in diameter,

Figs.24-32. High-power photomicrographs showing stages in spermatogenesis.

Fig.24. Testis in October containing spermatogonia showing mitotic figures. (X 1100).

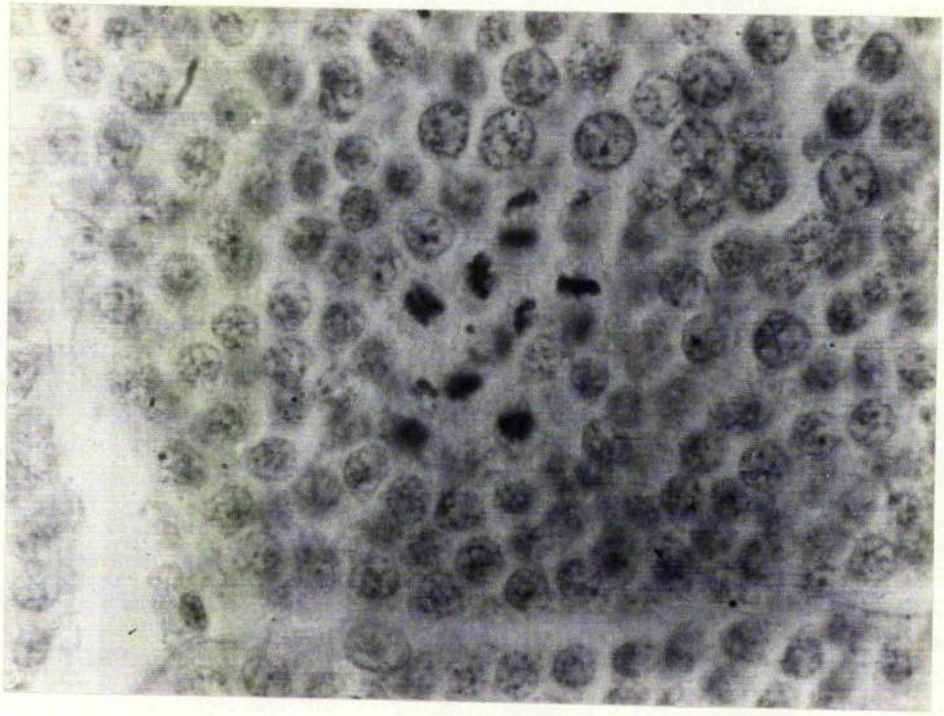
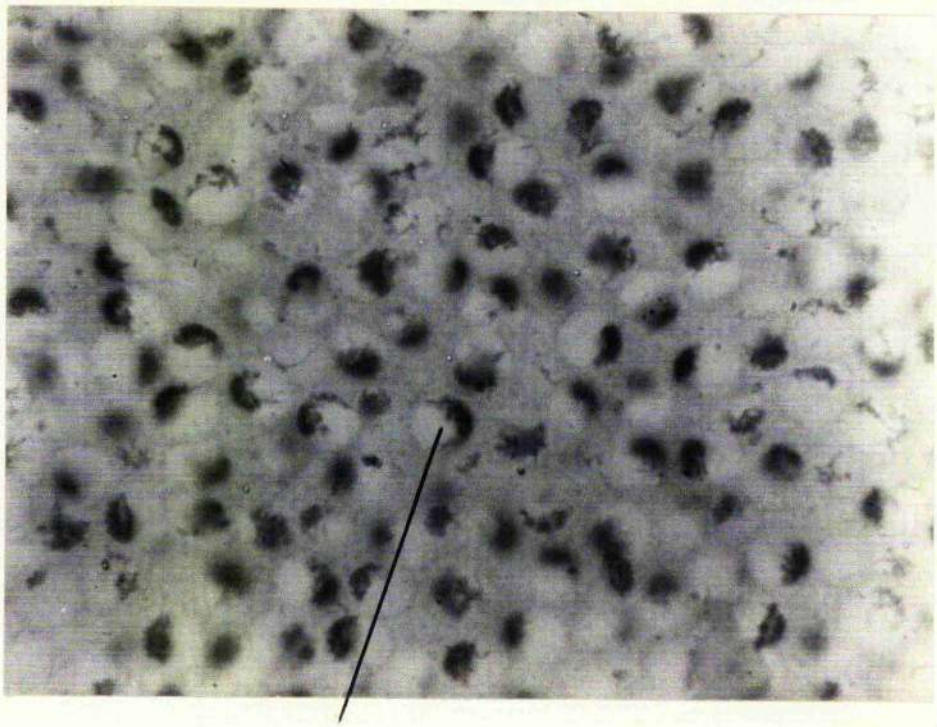


Fig.25. Testis in December containing primary spermatocytes in the leptotene stage of meiosis. (X 1100).

Fig.26. Testis in mid-February containing primary spermatocytes in the diakinesis stage of meiosis. (X 1100).



Leptotene

Diakinesis

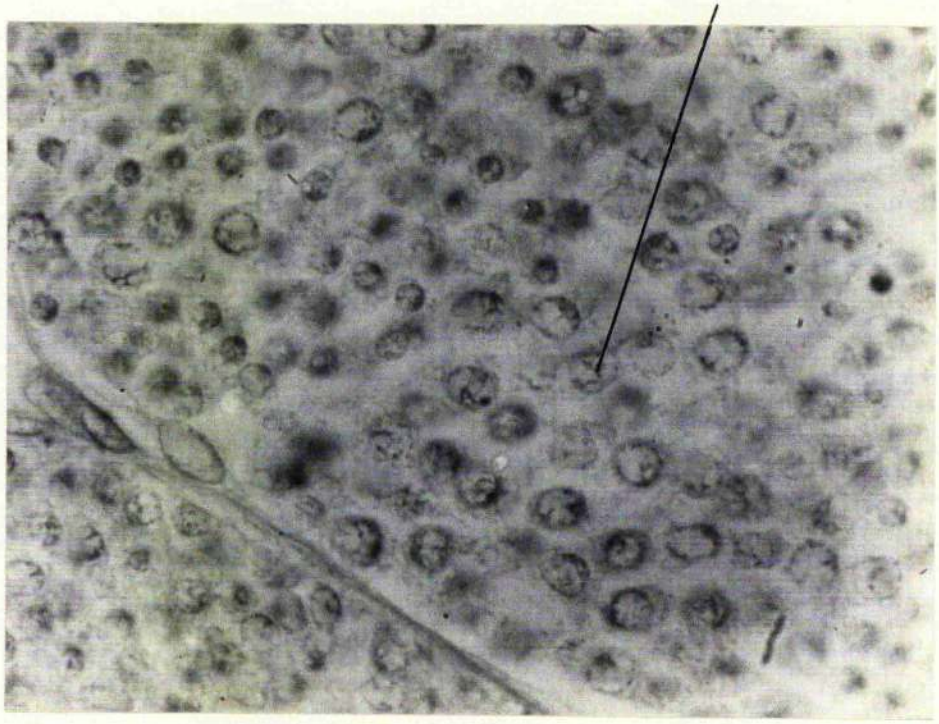


Fig.27. Testis in late February showing primary spermatocytes in diakinesis together with meiotic first metaphases. (X 1100).

Fig.28. Testis, late February, containing primary spermatocytes in diakinesis, meiotic first metaphases, secondary spermatocytes and second metaphases. (X 1100).

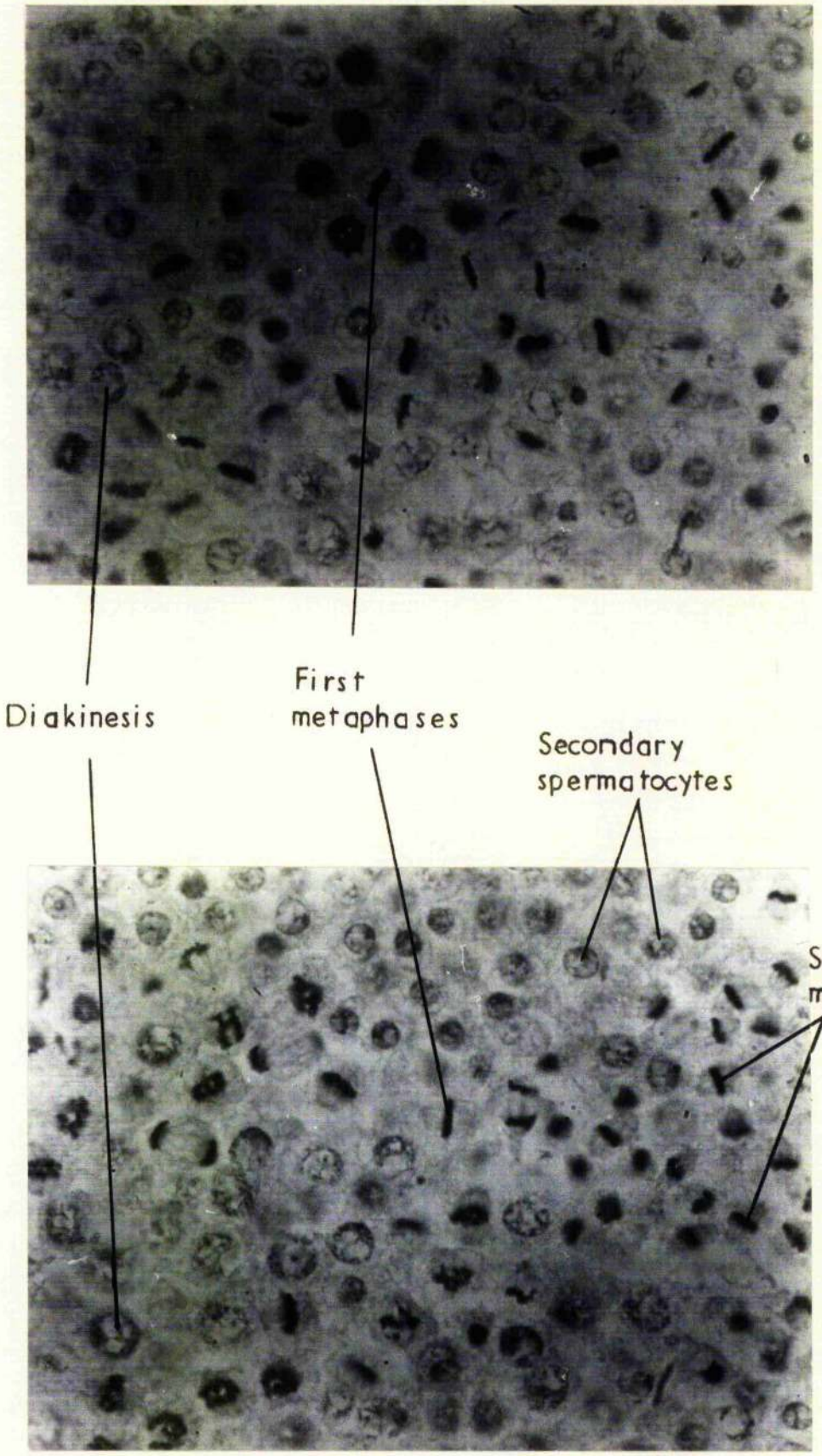

Diakinesis
First metaphases
Secondary spermatocytes
Second metaphase

Fig.29. Testis in early March, containing first metaphases, secondary spermatocytes, second metaphases and early spermatids. (X 1100).

Fig.30. Testis in early March, containing second metaphases and early spermatids. (X 1100).

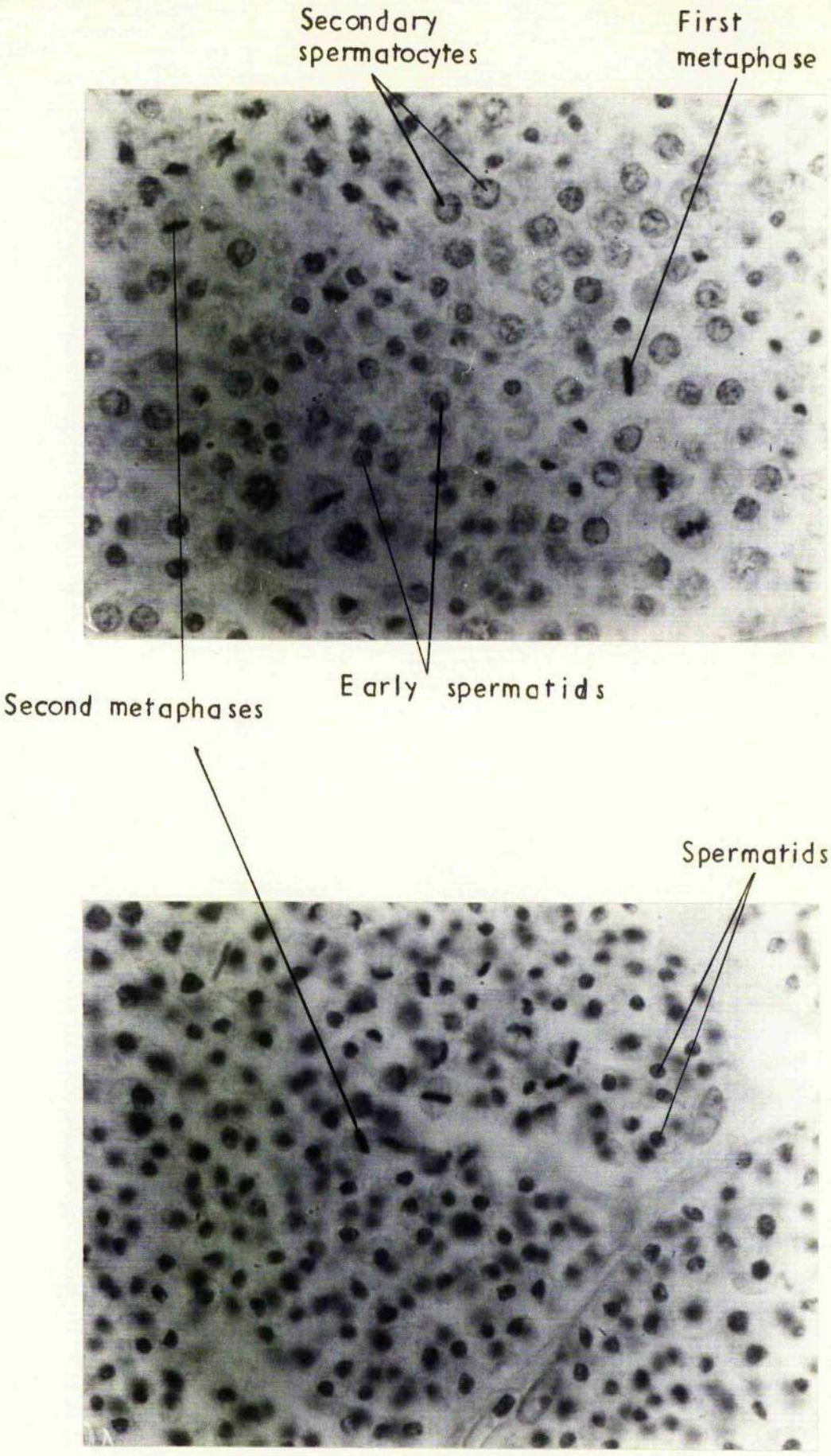
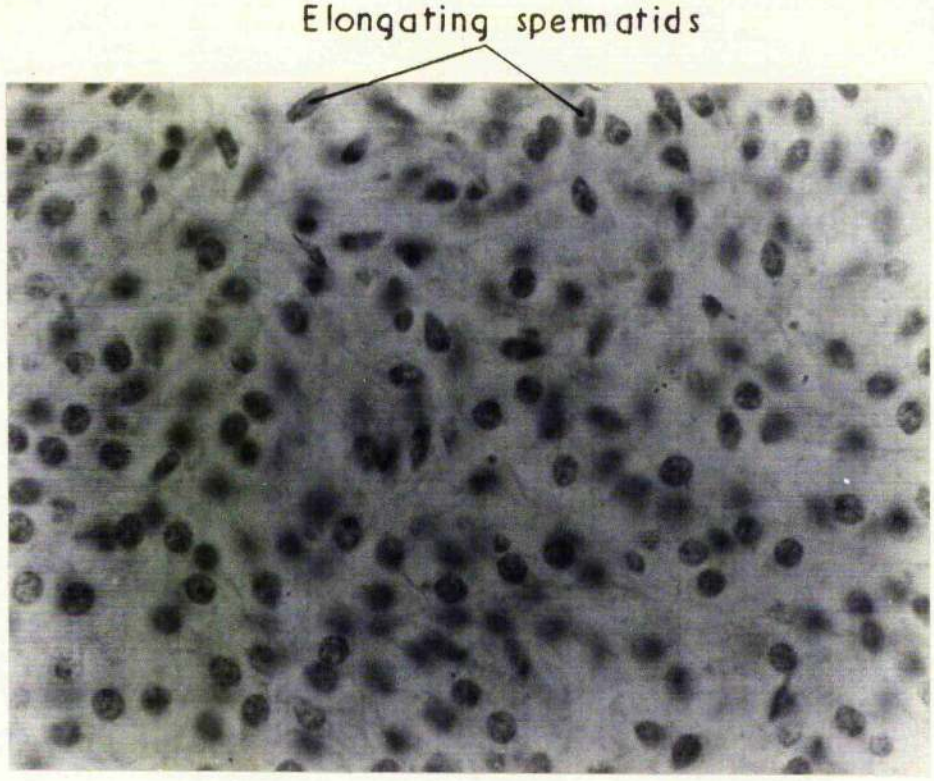
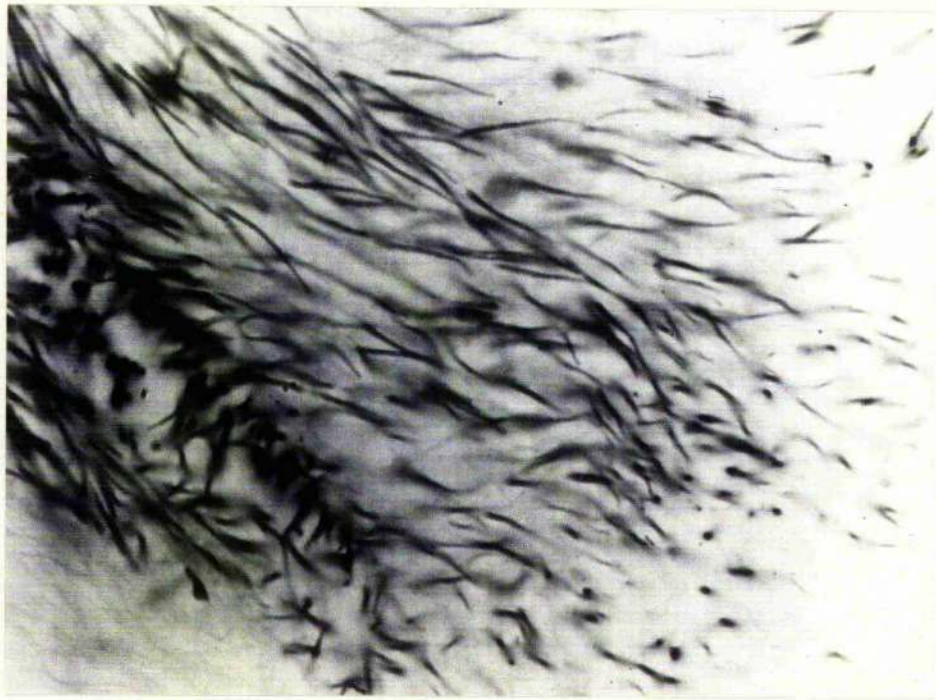

Secondary spermatocytes
First metaphase
Second metaphases
Early spermatids
Spermatids

Fig.31. Testis in mid-March showing elongation of spermatids. (X 1100).

Fig.32. Spermatozoa in mature testis in mid-April. (X 1100).


Elongating spermatids



with the chromosomes lying around the nuclear membrane. (Fig. 28 Secondary spermatocytes were seen much less frequently than other stages as they are short-lived, the second division of meiosis occurring very shortly after the first. Because of this, and the slight lack of synchrony between all the germ cells within a testis, examination in late February revealed all stages from primary spermatocytes in late prophase, to elongating spermatids. The spindles of the second division are longer (8μ) and of smaller diameter (4μ) than those of the first division, and can be positively identified since they occur, in many cases, in lobules containing, apart from the spindles, only spermatids (Fig. 30). As in the first metaphase, individual chromosomes cannot be recognised.

Spermatids.

Spermatids have extremely small nuclei, between 2 and 3μ in diameter, with a small quantity of cytoplasm (Fig. 31). After their formation, when chromosomes can still be resolved, the nuclei become more pycnotic and begin to elongate. Elongation continues throughout the maturation of the sperm, the heads of which ultimately reach approximately 14μ in length, and less than 1μ in diameter. (Fig. 32).

Spermatozoa.

Spermatozoa, when they are first formed in mid-March are randomly arranged within each lobule (Fig. 33). As

development proceeds, however, the sperm heads become clumped together generally to one side of the lobule, and the tails are whorled within the lobule (Fig.34.). In the mature testis many lobules become confluent, their walls breaking down prior to the release of the sperm into the body cavity.

ii) Lipid-containing cells.

Cells containing lipids demonstrable by the Sudan Black technique are found at the intersections of the lobule walls of the testis throughout the whole period of the spawni migration. From late September to March these are the only lipid-containing cells within the testis (Fig.35), but as the sperm matures lipids appear also within the lobule walls. The 'interstitial' cells stain deeply with Sudan Black and appear to contain a solid mass of lipid; the staining within the lobule walls is less intense, and the lipid is in the form of small droplets.(Fig.36).

The Schultz test for cholesterol and its esters was carried out in order to determine whether the lipids present in the 'interstitial' cells and the lobule boundary cells were steroids. A clear positive reaction was given by the 'interstitial' cells in all testis samples, from the beginning of the anadromous migration in late September until sexual maturity in April. The lobule boundary cells consistently gave negative results with the Schultz test in

Fig.33. Testis lobule in late March showing random arrangement of spermatozoa. (X 250).

Fig.34. Testis lobule in mid-April showing "whorling" of sperm. (X 250).

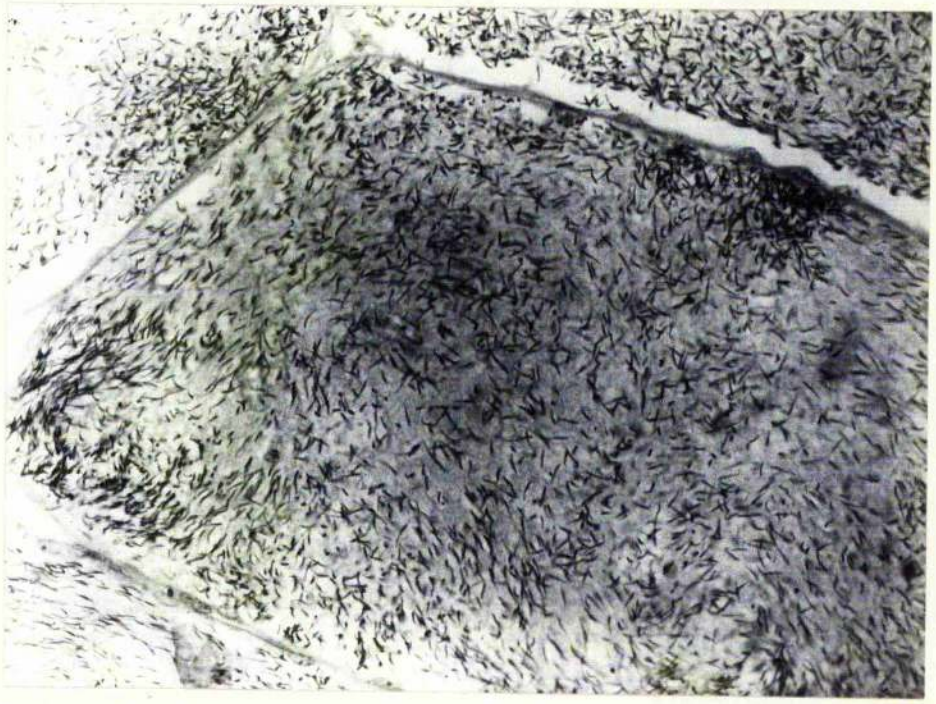


Fig.35. Section of immature testis stained with Sudan Black showing presence of lipids in "interstitial" cells. (X 250).

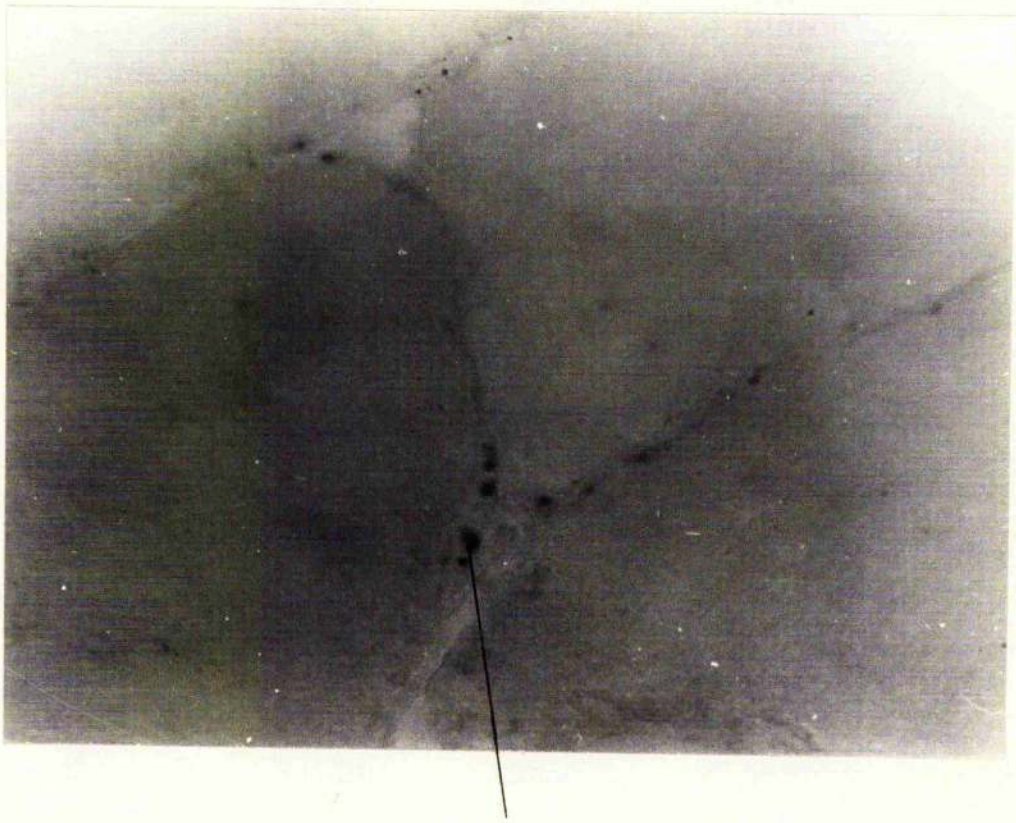
Fig.36. Sudan-stained section of mature testis showing small lipid droplets in cells of the lobule walls, as well as solid masses of lipid within the "interstitial" cells. (X 250).

Interstitial cells

Lobule-boundary cells

Fig.37. Section of testis fixed in October, showing positive Schultz reaction in the "interstitial" cells only. (X 250).

Fig.38. Section of mature testis fixed in mid-April, showing positive Schultz reaction in the "interstitial" cells only. (X 250).



Interstitial cells

intact animals (Figs. 37, 38).

The demonstration of Schultz-positive material in the 'interstitial' cells of the testis suggests that these cells may be the source of an androgenic steroid hormone. (But see effects of testosterone, p.77).

3. THE OVARY.

a) Review of literature.

Very few studies on the lamprey ovary have been recorded. Like his investigation of the testis, Okkelberg's (1921) work on ovary of the brook lamprey Entosphenus wilderi deals only with larvae up to the stage of sex differentiation

Okkelberg's description states that the germ cells of definitive female lampreys enter the prophase of the first meiotic division shortly after sex differentiation, several years before maturation takes place, and that the diplotene stage persists throughout the growth period, and probably up to the time of maturation, though he remarks that the study of nuclear detail during the growth period is difficult since the chromatin becomes scattered throughout the large nucleus.

The only cytological study which has been carried out on the oocytes of the adult lamprey is that of Chubareva (1957a, b), who found that the oocytes of Lampetra fluviatilis were ovulated at the metaphase stage of the second meiotic

division, and that maturation was completed after entry of the sperm.

Lanzing (1959) has given a description of the histology of the ovary of Lampetra fluviatilis, and the histological results presented on p.40 agree well with his findings.

Nowhere in the literature is there any reference to the occurrence of atretic follicles or corpora lutea in the lamprey ovary, though their occurrence in the hagfish Myxine glutinosa has been described by Lyngnes (1936). It should be noted here that there is a fundamental difference between the reproductive habits of the two groups of cyclostomes as the lamprey spawns once only and dies immediately after, while the hagfish has a life-cycle involving several breeding seasons.

b) Results of histological examination.

The single median ovary of the lamprey, suspended by the mesorchium, extends along the whole length of the body cavity. The ovary consists of numerous lamellae similar to those of the testis, each containing a large number of oocyte During the period of the spawning migration, oocytes account for by far the greater part of the volume of the ovary, a relatively small quantity of connective tissue being present. In October, at the beginning of the spawning migration, the ovary consists of a compact mass of oocytes bound together

by connective tissue. After ovulation, which occurs in late March, the oocytes become free from the connective tissue and are shed into the body cavity, whence they escape through pores into the urinogenital sinus and thus to the exterior.

Growth of the oocyte by increase in the yolk content occurs steadily between October and April, as shown by the graph of egg dry-weights (Fig.39). Each point on the graph is the mean of dry-weight of samples of 100 eggs from each of 3 lampreys. These weights showed an increase similar to the increase in egg size and ovary weight, determined over a similar period by Lanzing (1959).

Oocytes of the lamprey are elliptical in shape, the major diameter increasing from approximately 0.6 mm. in October to 1.1 mm. in April. The egg has clear animal and vegetative poles, the nucleus being found at the animal pole surrounded by the perinuclear cytoplasm, which contains no yolk platelets (Fig.40). The remainder of the egg is filled with yolk platelets of from 5 to 12μ diameter. The egg is contained within a double membrane, the zona radiata, which consists of the membrana interna and the membrana externa, both membranes exhibiting a very fine radial striation. These membranes are equal in thickness, and each increases slightly from 3μ in October to 4μ when ovulation occurs in April.(Fig.41

Each egg is enclosed within a follicle consisting of two cell layers - the inner granulosa, and the theca (Fig.41

The theca consists of a thin layer of flattened cells, completely enveloping the egg, generally about 4μ thick, with small, elongated nuclei. Small blood vessels are present among the cells of the theca, which appears to be the only vascularised layer of the egg. The granulosa is present only in the vegetative half of the egg, extending towards the anima pole just a little beyond the equator. The granulosa consist of a single layer of cells (Fig.42). When examined in Octobe the granulosa is about 10μ thick, the cells are extremely flattened and the cytoplasm contains little basophilic materia Beginning in January, the granulosa increases in thickness unt it reaches 25-30μ in April, when the cells are almost cuboidal (Fig.43). The cytoplasm of the granulosa cells becomes stron basophilic, staining with aniline blue, and the cells contain bodies similar in size and shape to the larger yolk platelets. Lanzing (1959) describes these bodies as acidophilic, but I found them to be chromophobic. No blood vessels are present within the granulosa.

Examination of histological sections of ovaries fixe during October and November revealed the presence of weakly basophilic vacuoles situated among the yolk platelets in the outer region of each egg. In an egg of 700μ diameter the vacuoles occurred in a layer approximately 100μ in depth (Fig.4 immediately beneath the zona radiata. Each vacuole was 20-30 in diameter. During December and January these vacuoles decre in size to 10-15μ diameter, and migrated closer to the zona radiat

Fig.39. Graph showing dry weights of samples of 100 eggs taken from lampreys between October and April. Each point represents the mean of 3 samples from different lampreys.

Dry wt. 100 eggs	mg.
Oct.13	7.1
Nov. 1	7.5
Nov.14	8.1
Dec. 4	8.7
Dec.20	9.8
Jan. 6	11.0

Dry wt. 100 eggs	mg.
Jan.15	12.6
Feb. 6	14.4
Feb.22	16.4
Mar. 1	18.2
Mar.24	21.0
Apl.13	25.0

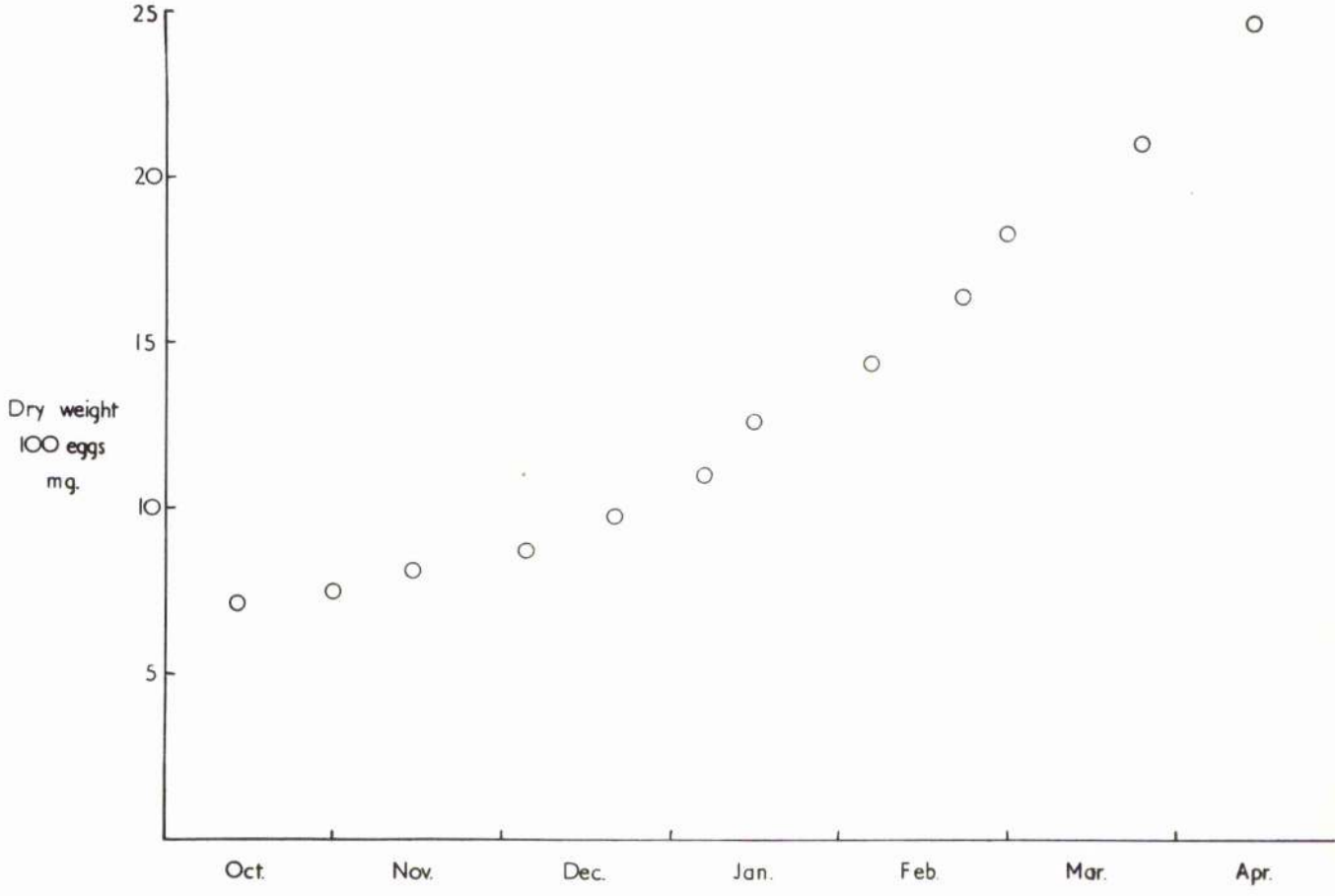

25
20
15
10
5
Dry weight
100 eggs
mg.
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.

Fig.40. Longitudinal section through immature egg in October. (X 250).

Fig.41. Higher power of vegetative pole of egg showing the zona radiata, granulosa and theca. (X 350).


Nucleus
Yolk platelets

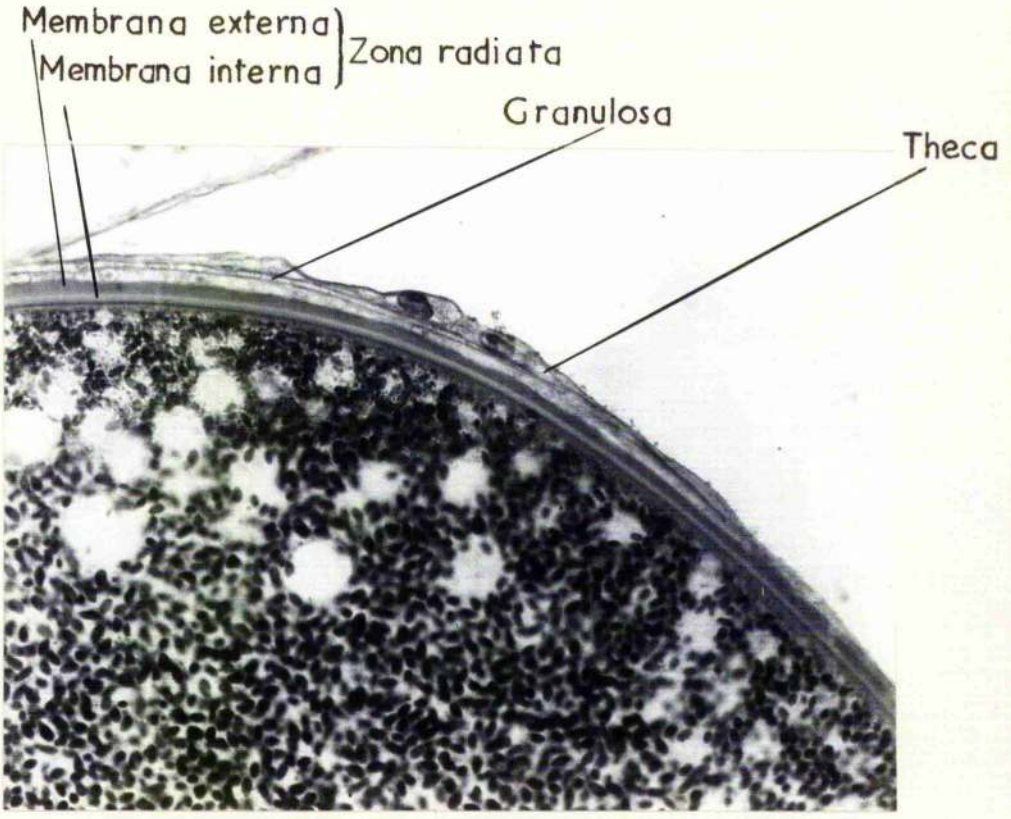

Membrana externa
Membrana interna
Zona radiata
Granulosa
Theca

Fig.42. Vegetative pole of immature egg in October showing squamous granulosa cells. (X 350).

Fig.43. Vegetative pole of mature egg in early April, when the cells of the granulosa have become almost cuboidal. (X 350).

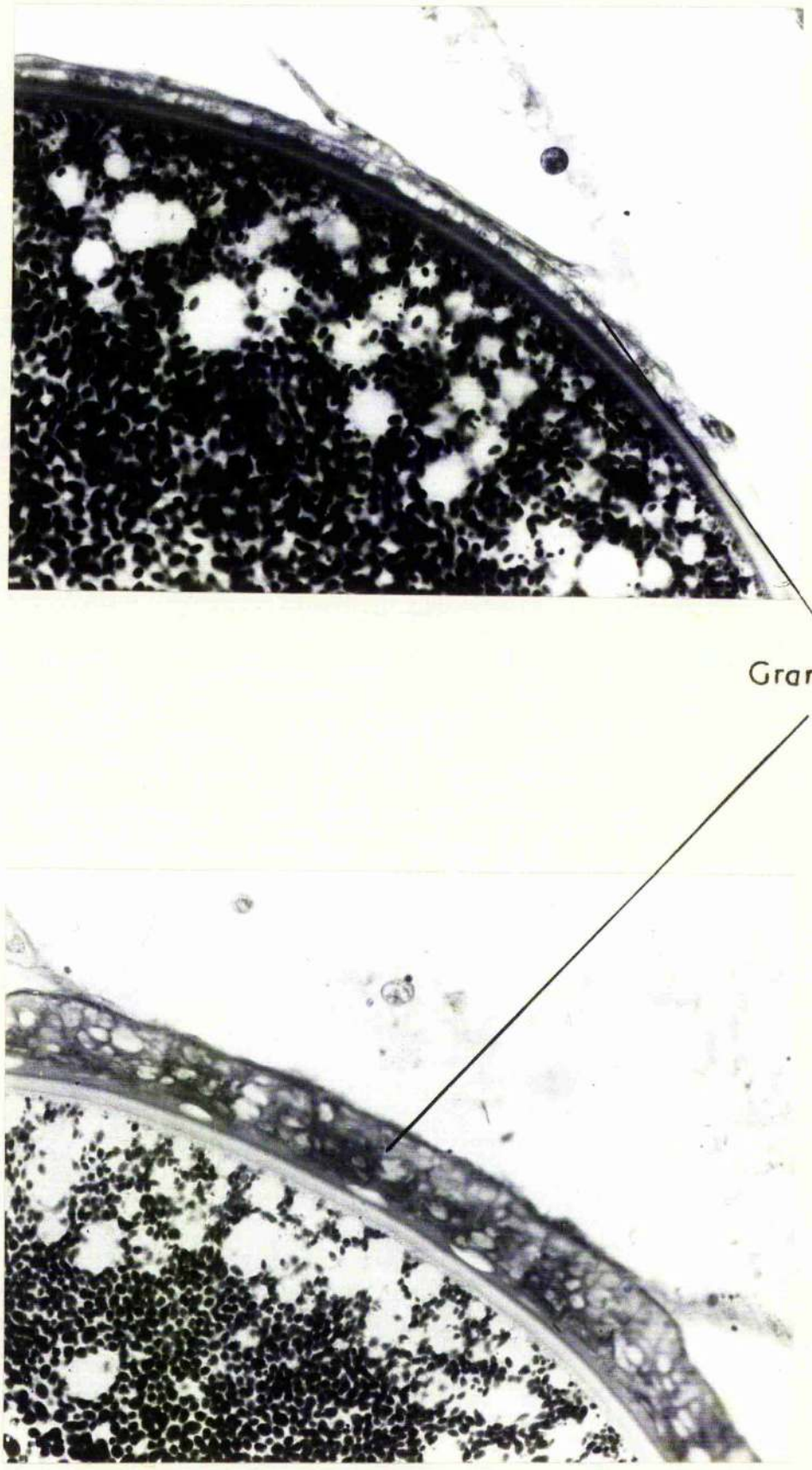
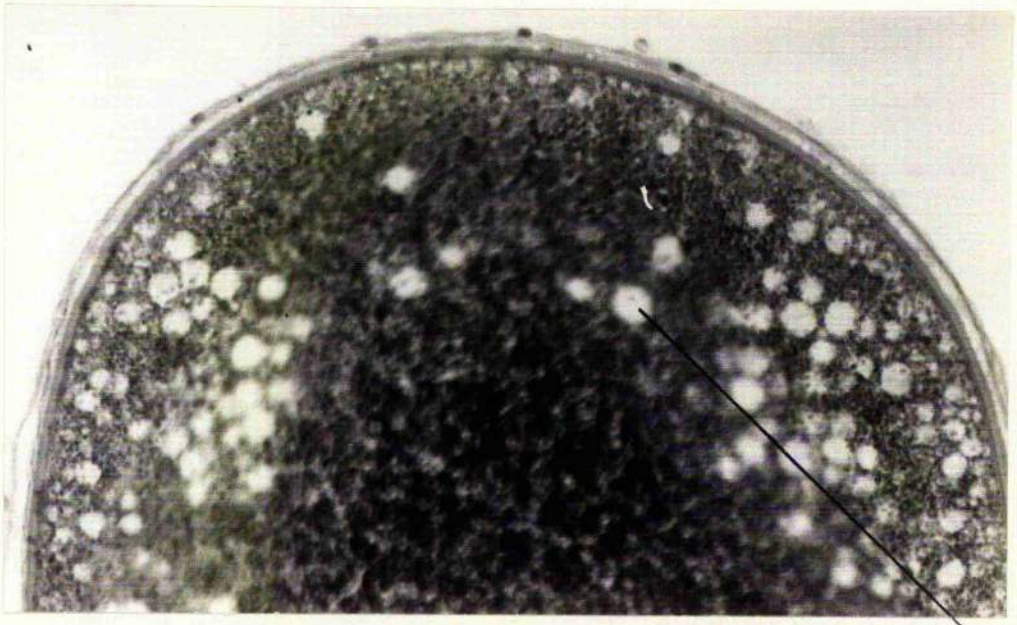

Granulosa

Fig.44. View of vegetative pole of immature egg in October, showing central alveoli present in a deep band. (X 200).

Fig.45. Vegetative pole of almost mature egg in late March, with central alveoli occupying a narrower layer. (X 200).

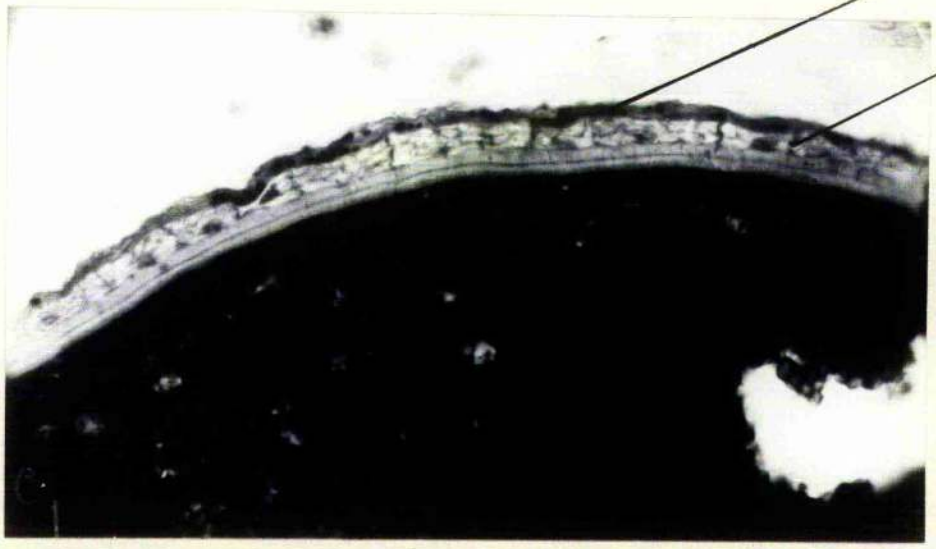
Fig.46. Vegetative pole of section of egg fixed in late March, stained with Sudan Black, showing presence of lipids in the theca. (X 200).



Alveoli

Theca

Granulos



occupying in the mature egg of approximately 1,000μ diameter, a layer 40μ deep (Fig.45). These vacuoles are the so-called cortical alveoli, probably responsible for the formation of the perivitelline space after fertilisation (Rothschild, 1958).

Application of the Sudan Black technique to histological sections of ovary cut in water-soluble wax showed that, apart from the obviously large quantity of lipids contained by the yolk, lipids were present in smaller quantiti also in the theca during the whole period of the spawning migration, from October to April (Fig.46).

The Schultz test for cholesterol produced a negative result, but it is perhaps questionable whether this is a true negative, because of difficulties with the technique. Treatment of sections of ovary tissue with the concentrated sulphuric acid and glacial acetic acid mixture employed in the Schultz test produced a general brown colouration of the tissues. It is possible that this colouration might have obscured the green colour, often faint, which denotes the presence of cholesterol or its esters in the Schultz reaction. Thus the possibility remains that the theca cells secrete an oestrogenic steroid hormone.

Unlike the ovaries of all other groups of vertebrate the ovaries of lampreys were never found to contain atretic follicles (corpora atretica) or corpora lutea. In other vertebrates, both corpora atretica and corpora lutea are

generally found. The former are produced by the *in situ* degeneration of oocytes which have passed a certain stage in development and may even be mature. This process presumably serves to remove unovulated mature oocytes from the ovary after spawning, in readiness for the maturation of new oocytes for ovulation during the next spawning season. Corpora lutea are the endocrine structures formed by invasion of the empty follicle by cells of the granulosa and theca.

In the lamprey all the oocytes present in the ovary mature and are ovulated simultaneously, very shortly before spawning occurs. Examination of the gonad of spent female lampreys shows that no oocytes are present, and that the empty follicles have not become organised into secretory structures similar to the corpora lutea of higher vertebrates. In view of the complete ovulation of all oocytes within the ovary, and the fact that the lamprey spawns only once in its lifetime and dies very soon after spawning, there is no possibility of the development of corpora lutea and no need for them from a functional point of view. As atretic follicles have not been observed in lampreys during their spawning migration it is probable that corpora atretica also do not occur in the river lamprey.

4. THE SECONDARY SEXUAL CHARACTERS.

On their re-entry into fresh water in September and October, the external appearance of male and female lampre is identical. The two dorsal fins are separated by a gap of usually more than 1 cm., with the cloaca hardly disturbing the smooth line of the ventral body surface. This condition is retained throughout the winter until the development of secondary sexual characters begins in March, reaching its climax in early April, when our stock of lampreys becomes sexually mature.

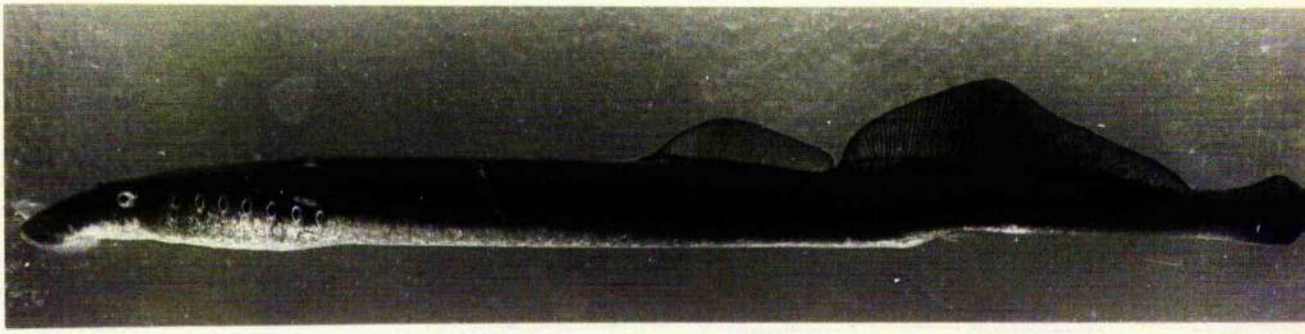
The development of the secondary sexual characters in the lamprey has been described by Young & Bellerby (1935) and Knowles (1939). In both sexes, during the onset of sexua maturity, the posterior dorsal fin enlarges and becomes swolle at its base; the fin extends forward to meet the anterior fin, and, especially in the male, radiating blood vessels become very prominent.

In the male (Figs.47, 48), the urinogenital papill enlarges to more than 1 cm. in length, the cloacal labia become hyperaemic and pores develop laterally in the wall of the urinogenital sinus, connecting body cavity with the exteri through the urinogenital papilla. Milt can be obtained from the papilla by gentle abdominal pressure from mid-March onward

In the female lamprey (Figs. 49, 50), the cloacal labia become extremely swollen, but there is no enlargement of the urinogenital papilla, which remains short, barely protrudir into the cloaca. As in the male, pores develop in the lateral walls of the urinogenital sinus, to conduct the eggs to the exterior. A post-anal fin develops, approximately 2 cm. long and 5 mm. deep, and the post-anal region of the tail curves upwards. Towards spawning time the abdomen of the female becomes swollen, due to enlargement of the eggs within, contributing to the marked difference between male and female lampreys when sexually mature.

Fig.47. Photograph of mature male lamprey showing secondary sexual characters. Note large vascularised second dorsal fin, swollen cloacal labia and extended urinogenital papilla.

Fig.48. Close-up of cloaca of mature male with urinogenital papilla extended with forceps.



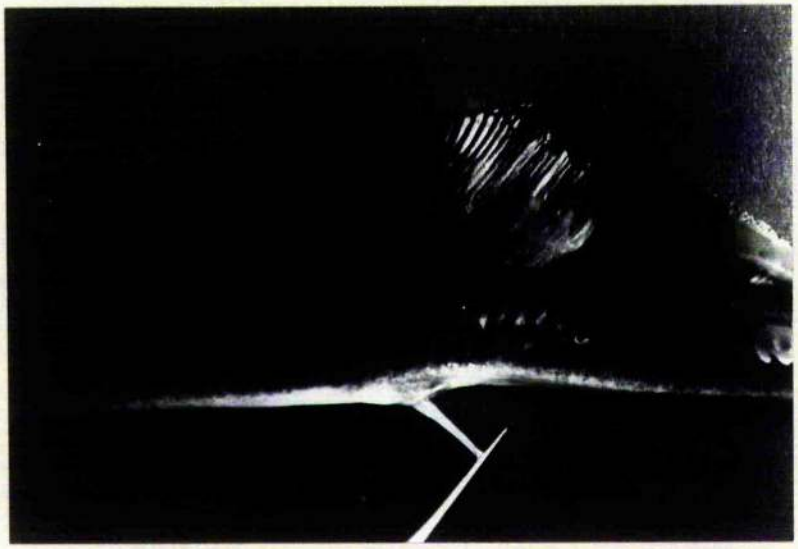
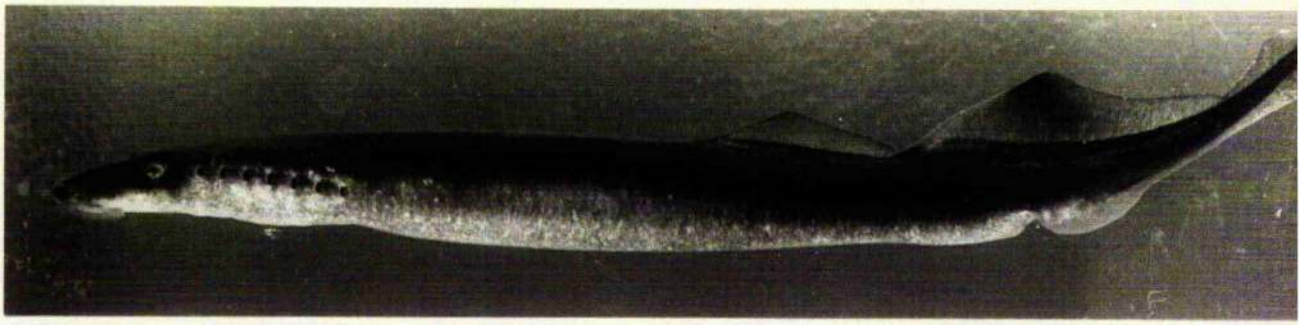
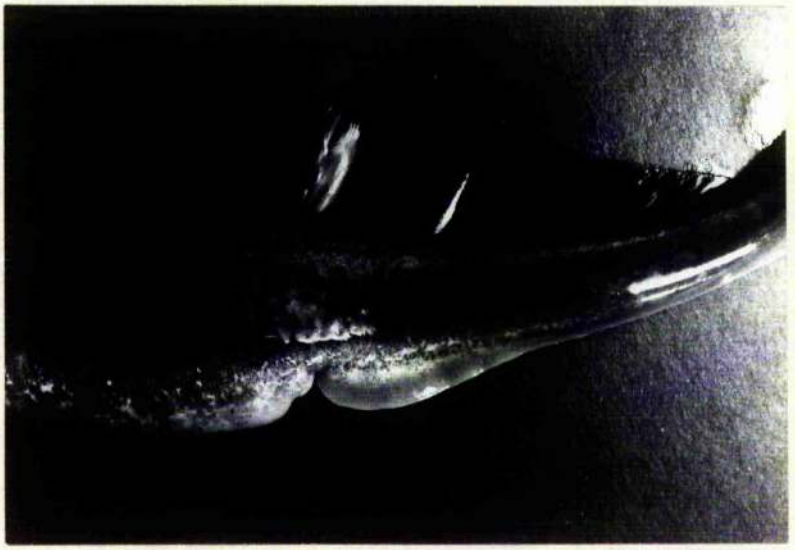


Fig.49. Photograph of mature female lamprey showing secondary sexual characters. Note the large second dorsal fin as in the male (Fig.47), the swollen cloacal labia, the post-anal fin and the abdomen distended with eggs.

Fig.50. Close-up of cloaca of mature female. No extensible urinogenital papilla is present (cf. Fig.48).





EXPERIMENTAL RESULTS.

1. HYPOPHYSECTOMY.

a) Review of Literature.

There are two references in the literature to hypophysectomy in cyclostomes, but the operation has never been investigated in the context of reproduction.

Young (1935) hypophysectomised ammocoetes and adults of Lampetra planeri and L.fluviatilis in his investigation of the control of the melanophores. Presumably long-term post-operative survival was not necessary for the colour-change experiments, and thus no observations on the effect of the operation on reproduction were made. Young found that removal of the pars intermedia (meta-adenohypophysis) together with the pars nervosa (neurohypophysis) caused the melanophores to contract, and the animals to remain maximally pale. The method of hypophysectomy described by Young is basically that which has been employed in this study.

Knowles (1941) refers to the examination of endostyle of hypophysectomised ammocoetes but gives no details of the technique of hypophysectomy. As the animals used in Knowles' work were not of reproductive age, no results were obtained concerning the effect of hypophysectomy on reproduction.

b) Experimental results.

After the operation of hypophysectomy survival of the lampreys was excellent and their susceptibility to fungal infection generally little greater than that of normal lampreys

The first effect of hypophysectomy, noticeable after less than 24 hours, was the paling of the skin due to contracti of the melanophores. The hypophysectomised lampreys were rath less active than intact animals, and were less inclined to make use of the sucker for attaching themselves to the side of the tanks when resting, even though great care had been taken to avoid undue damage to the sucker muscle during the operation.

Normal lampreys become sexually mature during April when, in the wild, they spawn and die shortly after. In fact, lampreys of the laboratory stock did not spawn, due no doubt to the unnatural environment in the tanks, but death occurred in April or May. No normal lampreys survived beyond May. The hypophysectomised lampreys on the other hand developed no secondary sexual characters and thus could not spawn even given natural conditions, and they were not subject to the natural death which normally follows spawning. Some of the hypo-physectomised lampreys were killed in April when normal lamprey were mature; a considerable number of the lampreys allowed to survive beyond April were still in good condition when killed in May, June or July, and several survived into August, September and even October. It is possible that the pituitary

is responsible for the initiation of metabolic changes in preparation for spawning, changes which do not permit prolonged survival. In the hypophysectomised lampreys survival appears to be limited only by the extent of the food reserve.

In this work conclusions have been drawn only from results obtained from animals which survived the operation in good condition. These have, however, been supplemented from lampreys which died, where the interval between death and autopsy did not exceed 12 hours and was generally considerably less than this.

Serial sections were cut and stained for about a fifth of the hypophysectomised lampreys, and for any with anomalous results. Special attention was paid to the lateral regions of the meso-adenohypophysis when examining the sections None of the lampreys included in this section on the effects of hypophysectomy contained any pituitary remnants. For the results of partial hypophysectomy see p. 79.

i) Effects on the testis.

Effects on spermatogenesis.

Preliminary experiments already published (Dodd, Evennett & Goddard, 1960) indicated that the testes of lampreys hypophysectomised in January contained sperm when examined in April. Subsequent experiments have confirmed this and extended the findings.

TABLE I. Results of Hypophysectomy of male lampreys.

Date of operation	Lamprey No.	Date killed	State of sec. sex at death	Stage of germ cells at hypo.	Stage of germ cells: April		May		June		July	
Oct.14	1	Jun.22	None	S'gonia	14	Sc 1 Lept Sc 1 Diak	14		22	Sperm		
14	2	Jun.24	None	S'gonia	14	Sc 1 Diak			24	Sperm		
20	3	Jul. 4	None	S'gonia	14	Sc 1 Diak S'cytes 2			24	Sperm	4	Sperm
25	4	Jun.17	None	S'gonia					17	Sperm		
25	5	May 27	None	S'gonia	14	Sc 1 Diak	27	Sperm				
25	6	Jun.27	None	S'gonia	14	Sc 1 Diak			27	S'tids		
25	7	May 15	None	S'gonia	26	S'tids Sperm	15	Sperm				
25	8	Jul.19	None	S'gonia	14	Sc 1 Diak			24	Sperm	19	Sperm
Nov. 6	9	Jun.20	None	Sc 1 Lept	13	Sc 1 Diak S'cytes 2 S'tids			20	Sperm		
8	10	Apl.13	None	S'gonia	13	S'cytes 2 S'tids						
8	11	Apl.13	None	S'gonia	13	Sc 1 Diak						
8	12	Apl.13	None	Sc 1 Lept	13	Sc 1 Diak S'cytes 2 S'tids						
14	13	May 18	None	Sc 1 Lept	14	S'cytes 2 S'tids	18	S'tids Sperm				
14	14	Apl.13	None	Sc 1 Lept	13	Sc 1 Diak						
19	15	Apl.14	None	S'gonia	14	Sc 1 Diak S'cytes S'tids						
19	16	Apl.14	None	S'gonia	14	Sc 1 Diak						
29	17	May 25	None	S'gonia			25	Sperm				
29	18	May 8	None	Sc 1 Lept	13	Sc 1 Diak	8	Sc 1 Diak S'cytes 2 S'tids				
Dec. 5	19	May 8	None	Sc 1 Lept	15	Sc 1 Lept Sc 1 Diak	8	Sc 1 Diak S'cytes 2 S'tids				
8	20	Jun.10	None	Sc 1 Lept					10	Sperm *		
11	21	May 12	None	Sc 1 Lept			12	Sperm *				
11	22	May 3	None	Sc 1 Lept	14	S'tids	13	Sperm				
11	23	Jul. 3	None	Sc 1 Lept			24	Sperm			3	Sperm
11	24	May 20	None	Sc 1 Lept			20	Sperm				
11	25	May 5	None	Sc 1 Lept			5	Sperm				
11	26	May 12	None	Sc 1 Lept	14	Sc 1 Diak	12	S'tids Sperm				
11	27	May 11	None	Sc 1 Lept			11	Sc 1 Diak S'cytes 2 S'tids				
12	28	May 8	None	Sc 1 Lept			8	Sperm				
14	29	Jun. 6	None	Sc 1 Lept	14	S'tids			6	Sperm		
14	30	Jul.12	None	Sc 1 Lept	13	S'tids	15	Sperm			12	Sperm *
14	31	May 8	None	Sc 1 Lept	14	Sc 1 Diak S'cytes 2	8	S'tids Sperm				
14	32	May 12	None	Sc 1 Lept			12	S'tids Sperm				
29	33	May 9	None	Sc 1 Lept			9	Sperm *				
29	34	May 7	None	Sc 1 Lept	13	Sperm	7	Sperm *				
29	35	May 22	None	Sc 1 Lept			22	Sperm				
29	36	Jun.21	None	Sc 1 Lept			24	Sperm	21	Sperm		
29	37	May 5	None	Sc 1 Lept			5	Sperm *				
29	38	May 16	None	Sc 1 Lept			16	Sperm *				
29	39	May 21	None	Sc 1 Lept			21	Sperm				

TABLE I (continued).

Date of operation	Lamprey No.	Date killed	State of sec. sex at death	Stage of germ cells at hypo.	Stage of germ cells: April		May		June		Jul
Jan.17	40	May 15	None	Sc 1 Lept			15	Sperm *			
23	41	Jun. 2	None	Sc 1 Lept					2	Sperm **	
24	42	May 30	None	Sc 1 Lept			30	Sperm *			
30	43	May 15	None	Sc 1 Lept			15	Sperm *			
30	44	Apl.25	None	Sc 1 Lept	25	Sperm					
30	45	Apl.28	None	Sc 1 Lept	28	Sperm *					
30	46	June13	None	Sc 1 Lept					13	Sperm **	
30	47	Jun.13	None	Sc 1 Lept Sc 1 Diak					13	Sperm **	
30	48	Apl. 8	None	Sc 1 Lept	8	Sperm					
30	49	Apl. 9	None	Sc 1 Lept Sc 1 Diak	9	Sperm *					
Feb. 8	50	May 10	None	Sc 1 Diak S'cytes 2 S'tids			10	Sperm **			
8	51	Apl.14	None	Sc 1 Diak S'cytes 2 S'tids	14	Sperm *					
Mar.14	52	Jun.27	None	S'tids					27	Sperm **	
15	53	Jun.25	None	S'tids					25	Sperm *	
20	54	Jun. 3	Slight	S'tids					3	Sperm **	
20	55	Oct.12	None	S'tids							Spe (Oc
20	56	Sep.30	None	S'tids							Spe (Se

S'gonia - Spermatogonia

Sc 1 Lept - Primary spermatocytes in leptotene

Sc 1 Diak - Primary spermatocytes in diakinesis

S'cytes 2 - Secondary spermatocytes

S'tids - Spermatids

Sperm - Spermatozoa not whorled

Sperm * - Spermatozoa slightly whorled

Sperm ** - Spermatozoa more whorled, almost as in normal lamprey

Fifty-six male lampreys were hypophysectomised between October and March. The accompanying Table shows the stages reached by the germ cells as determined by biopsy at the time of operation, and examination after the animals had been killed. In some cases results of additional biopsy samples taken around the normal spawning time are included.

Use of the biopsy technique in an investigation of this kind appears to be almost unique, and is of considerable value since it provides the only way of knowing with certainty the stage which spermatogenesis has reached in an animal at any time. The large lobular testis of the lamprey is, of course, ideal for this technique.

Biopsy samples of testes were taken in April from seven of the eight surviving lampreys hypophysectomised in October. All but one were taken on April 14th; of these one contained primary spermatocytes in both leptotene and diakinesis, four contained primary spermatocytes in diakinesis only, and one contained primary spermatocytes in diakinesis, first meiotic metaphases, and secondary spermatocytes. A biopsy sample taken from lamprey no.7 on April 26th showed that spermatids and early sperm were present.

Further examination of the testes of the animals, by biopsy, or after death, showed that, except for lamprey no.6, spermatozoa were present in May, June and July.

The microscopic structure of these sperm appeared to be normal, but they were never whorled within the lobule in the manner of mature sperm from a normal lamprey. It was not possible to test the fertilising capacity of the sperm fro these animals as it was not formed until some months after normal female lampreys had reached maturity and subsequently died. Lamprey no.6 was killed on June 27th, when its germ cells were still at the stage of spermatids. The spermatids in this case were accompanied by pycnotic nuclei, the occurrence of which is discussed on p. 96 (Fig.51).

Examination in April of the testes of lampreys hypophysectomised in November revealed the presence of primary spermatocytes in diakinesis in four cases (nos.11, 14, 16, 18), primary spermatocytes in diakinesis together with secondary spermatocytes and spermatids in three cases (nos.9, 12, 15), and secondary spermatocytes and spermatids in two animals (nos.10 and 13). Four lampreys hypophysectomised in November survived beyond April, no.13 containing spermatids and sperm and no.18 containing stages up to spermatids when examined in May. Unwhorled sperm were present in no.17 (May) and no.9 (June). It is probable that, had those lampreys which contai spermatids in April survived longer, spermatozoa would have developed by May or June.

Lampreys hypophysectomised in December and examined by biopsy in April were found to contain late primary spermato-

cytes (nos.19 and 26), late primary and secondary spermatocyte (no.31), spermatids (nos.22, 29, 30) and spermatozoa (no.34). When examined in May, animals nos.19 and 27 showed primary spermatocytes in diakinesis, secondary spermatocytes and spermatids. Spermatozoa were present in seventeen lampreys when examined in May, slightly whorled in five of these, and accompanied by spermatids in three cases. Three lampreys hypophysectomised in December survived into June, and two into July. All five contained sperm, but slight whorling was seen in only one of each group (nos.20 and 30).

All lampreys hypophysectomised in January and later and examined in April developed sperm. In two of the four lampreys hypophysectomised in January and killed in April the sperm showed slight whorling. The three lampreys hypophysectomised in January and examined in May showed slight whorling, and the three examined in June contained sperm almost as tight whorled as in the normal lamprey. The sperm of lamprey No.51 hypophysectomised in February, showed some whorling on examination in April, and that of no.50 was tightly whorled in May. Of the lampreys hypophysectomised in March, two contained tightly whorled sperm, and one slightly whorled, in June.

It appears from these results that spermatozoa can be formed in the absence of the pituitary in the lamprey, though spermatogenesis proceeds at a slower rate. Thus

Fig.51. High-power photomicrograph of testis of lamprey no.6 showing pycnotic nuclei among spermatids. (X 1100).

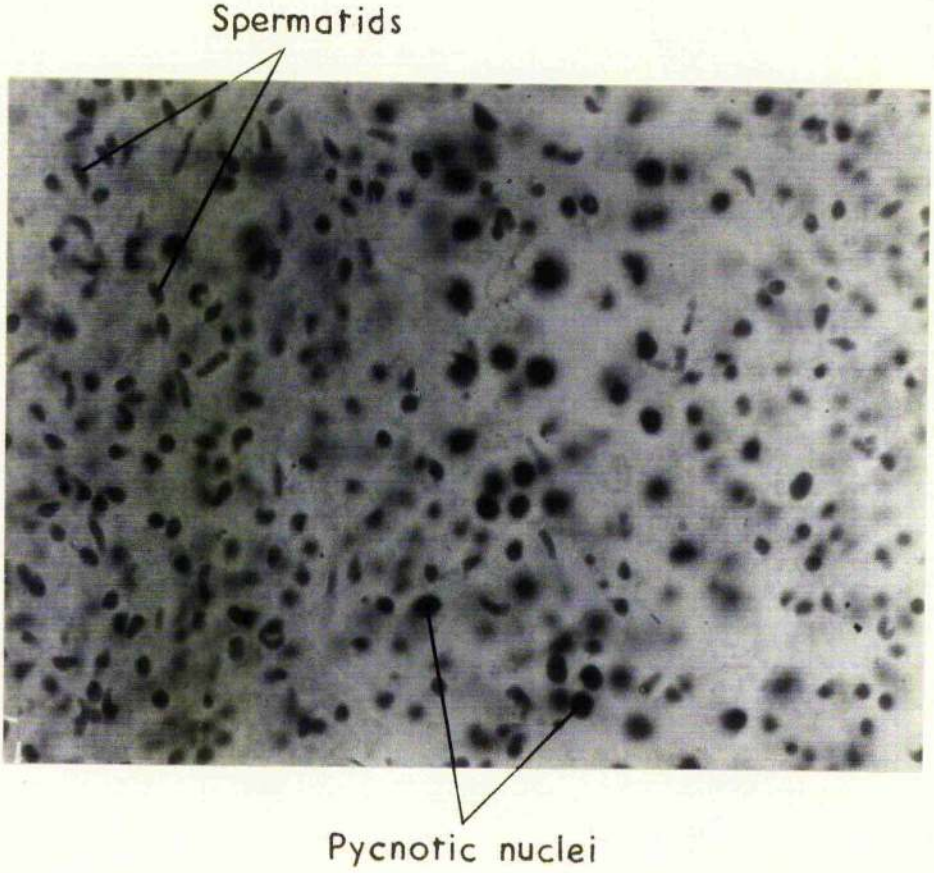
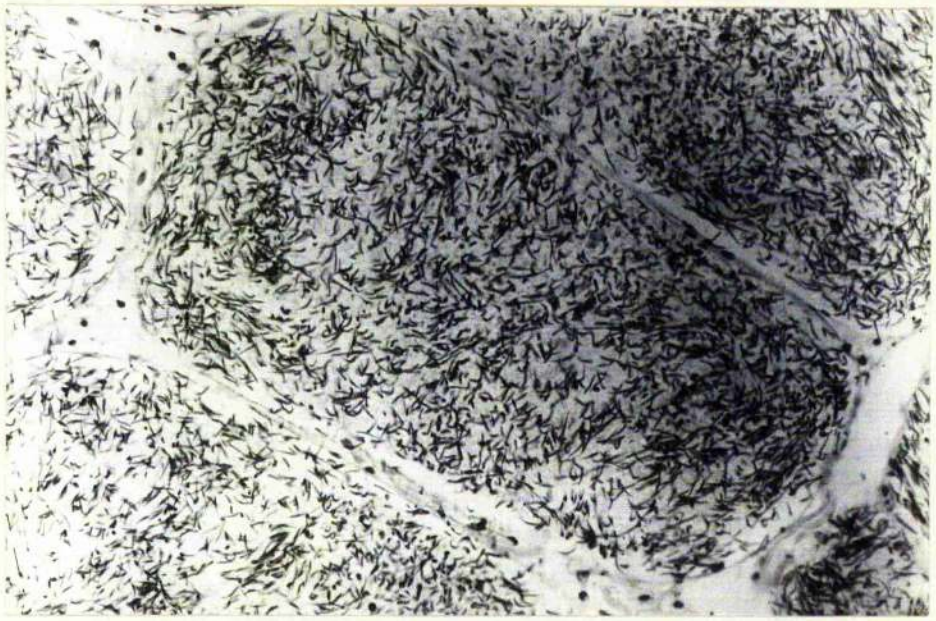
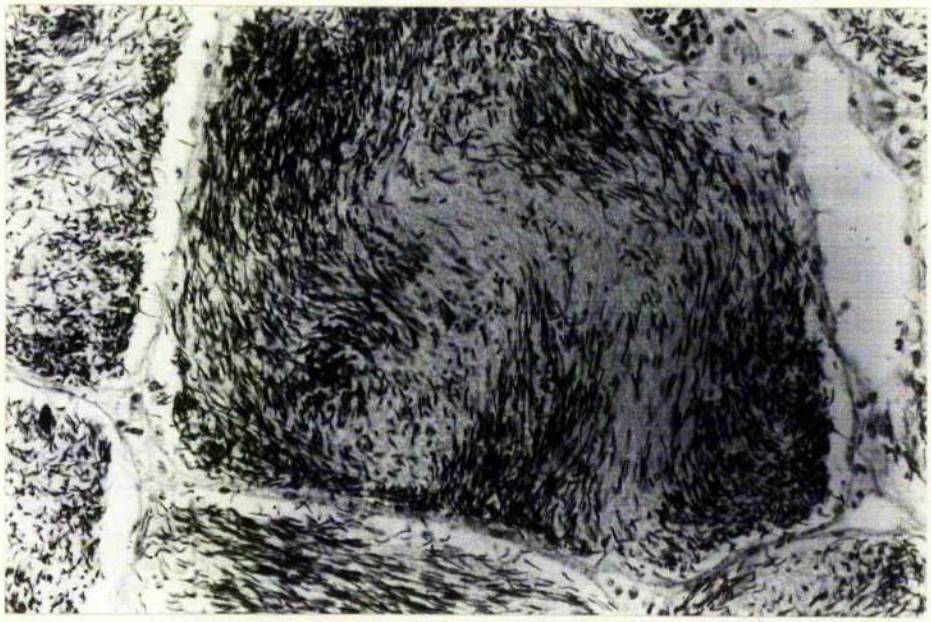

Spermatids
Pycnotic nuclei

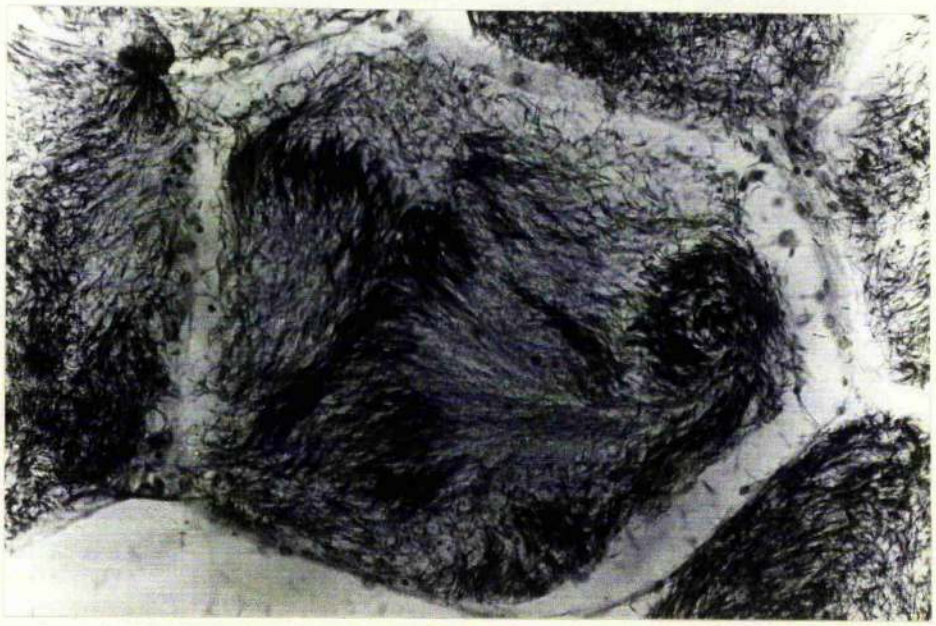
Fig.52. Lobule of testis of lamprey no.34, hypophysectomised in December and examined in April, showing randomly-arranged sperm. (X 250).

Fig.53. Lobule of testis of lamprey no.45, hypophysectomised in January and examined in April, showing slight "whorling" of sperm. (X 250).

Fig.54. Testis lobule of lamprey no.41, hypophysectomised in January and examined in June, showing almost normal "whorling" of sperm. (X 250).







hypophysectomy performed when the germ cells are at the stage of spermatogonia (in October) causes more delay in formation of sperm than hypophysectomy in December of January when the prophase of the first meiotic division is in progress Hypophysectomy in February or March, when secondary spermatocytes and spermatids are present, causes little disturbance of the timing of spermatogenesis.

While lampreys hypophysectomised at any time appeared to be able ultimately to complete spermatogenesis, the sperm was not usually whorled in the normal manner. No sign of whorling was observed in any lamprey hypophysectomised in October or November, even when examined as late as June or July. In the lampreys hypophysectomised in December, slight whorling was found in seven out of the twenty lampreys which contained sperm in May, June or July. Only one lamprey hypophysectomised in December had developed sperm by April, and in this case no sign of whorling was evident (Fig. 52). All of the seventeen lampreys hypophysectomised in January and later developed sperm, and in only two cases (nos.44 and 4 examined in April) was there no sign of whorling. Slight whorling was seen in seven (Fig. 53) and the remaining eight were more whorled but never quite as intensely as in normal lampreys (Fig. 54). It appears that whorling is more normal in the lampreys in which hypophysectomy was carried out later, and in those which were allowed to survive longer. Some of

the lampreys hypophysectomised in late January, February and March and examined during May or June showed a nearly normal testis picture. It is possible that whorling might eventually have occurred in the testes of all the hypophysectomised lampreys had they been able or allowed to survive for a sufficiently long time after pituitary removal.

Effects on lipid content.

In mature intact lampreys, application of the Sudan black technique to sections cut in water-soluble wax indicated the presence of lipids within the 'interstitial' cells, and in the form of small droplets within the cells of the lobule walls (Fig. 55). Sudan-stained sections of testis from hypophysectomised lampreys were examined, material in some cases being obtained from biopsy samples taken at the time of sexual maturity of normal lampreys or, more frequently at autopsy at a time when spermatogenesis was complete. Hypophysectomy appeared to have little effect on the lipid distribution and content of the lamprey testis (Fig. 56), whether the operation was performed in October or as late as March, though the appearance of lipids in the lobule walls was delayed until the formation of spermatozoa.

In biopsy samples taken in April from lampreys nos.1, 2 and 3, hypophysectomised in October, the germ cells had reached the stage of primary spermatocytes. At this time lipids were present in the 'interstitial' cells and,

to a slight extent, in the cells of the lobule walls; the appearance of the testis at this stage was identical to that of the normal testis examined in early March with respect to the germ cells and the lipid distribution. By May, June or July, spermatozoa were formed in the testes of lampreys hypophysectomised in October, November and December. Sudan staining of the testes at this time revealed a lipid distribut identical to that of the mature lamprey testis, the 'interstit cells staining deeply, and small lipid droplets being disperse within the cells of the lobule walls. In lampreys hypophysectomised later than those described above, in January, February and March, spermatozoa were present in April, hypophysectomy having caused less delay in spermatogenesis. In these lampreys lipids were present in the lobule walls on examination in April. The appearance of lipids within the lobule walls thus appears to be subject to a similar retardati to that imposed on spermatogenesis by hypophysectomy.

The Schultz reaction for cholesterol and its esters gave a positive result for the lipids of the 'interstitial' cells, and was negative for those in the lobule walls, in hypophysectomised as well as normal lampreys (Fig.57).

Sham-operated lampreys (Table II) in which the pituitary was left intact were identical in every respect to normal unoperated lampreys, showing mature secondary sexual characters and tightly whorled sperm in April and May.

Fig.55. Section of mature testis fixed in April and stained with Sudan Black, showing lipids present in the "interstitial" cells and the lobule-boundary cells. (X 250).

Fig.56. Section of testis of lamprey no.34, hypophysectomised in December and fixed in May, showing lipid-distribution similar to normal mature testis (Fig.55. (X 250).

Interstitial cells

Lobule-boundary cells

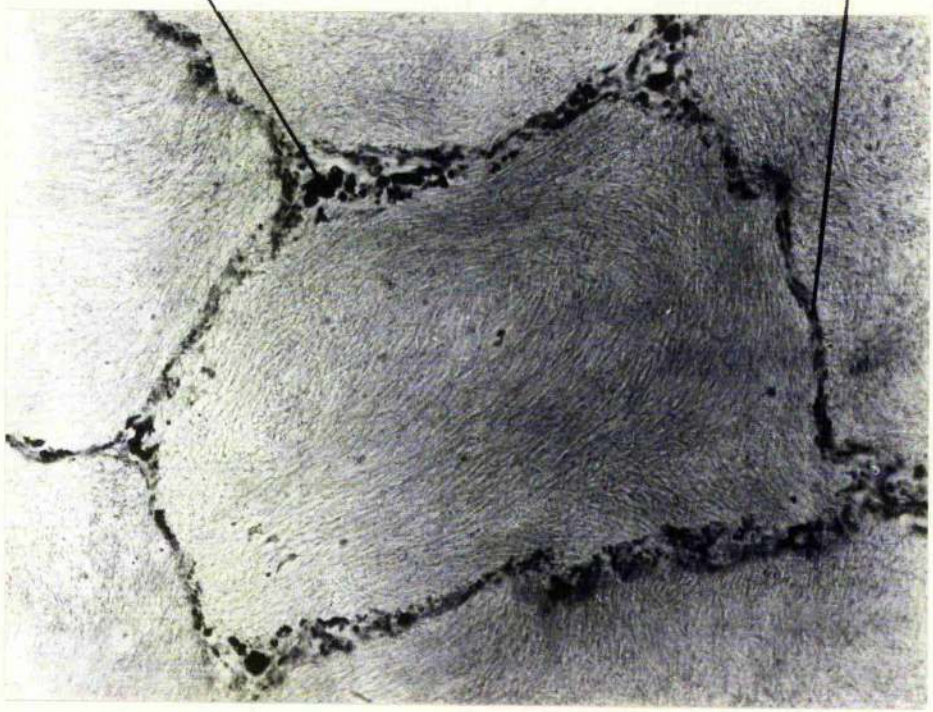
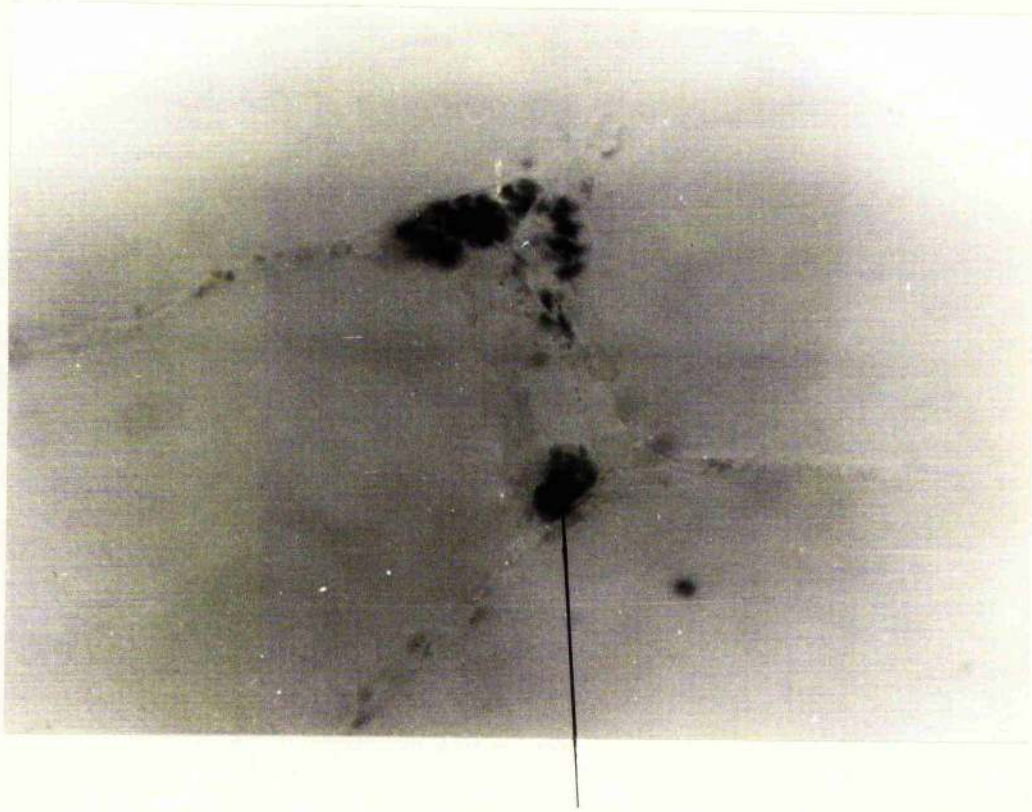


Fig.57. Positive Schultz reaction in "interstitial" cells only, in testis of hypophysectomised lamprey (cf. Figs.37 and 38). (X 250).



Interstitial cell

TABLE II. Results of Control Hypophysectomy of male lampreys

Date of operation	Lamprey No.	Date killed	State of sec. sex at death	Stage of germ cells at hypo.	Stage of germ cells: April		May		June	
Oct.19	57	May 5	Mature	S'gonia			5	Sperm **		
24	58	May 5	Mature	S'gonia			5	Sperm **		
25	59	May 7	Mature	S'gonia			7	Sperm **		
25	60	Apl.25	Mature	S'gonia	25	Sperm **				
Nov. 9	61	Apl.20	Mature	Sc 1 Lept	20	Sperm **				
9	62	May 5	Mature	S'gonia			5	Sperm **		
Dec. 8	63	Apl.23	Mature	Sc 1 Lept	23	Sperm **				
14	64	May 6	Mature	Sc 1 Lept			6	Sperm **		
18	65	May 3	Mature	Sc 1 Lept			3	Sperm **		
Jan.17	66	Apl.20	Mature	Sc 1 Lept	20	Sperm **				
20	67	May 7	Mature	Sc 1 Lept			7	Sperm **		
29	68	Apl.20	Mature	Sc 1 Lept Sc 1 Diak	20	Sperm **				
Feb.15	69	May 25	Mature	Sc 1 Diak S'cytes 2 S'tids			25	Sperm **		
15	70	Apl.24	Mature	Sc 1 Diak S'cytes S'tids	24	Sperm **				
Mar.14	71	Apl.24	Mature	S'tids	24	Sperm **				
14	72	Apl.29	Mature	S'tids	29	Sperm **				

S'gonia - Spermatogonia

Sc 1 Lept - Primary spermatocytes in leptotene

Sc 1 Diak - Primary spermatocytes in diakinesis

S'cytes 2 - Secondary spermatocytes

S'tids - Spermatids

Sperm ** - Spermatozoa more whorled, almost as in normal lamprey

(ii) Effects on the ovary.

The results of hypophysectomising thirty-one female lampreys are analysed in Table III, and these may be compared with the results of control hypophysectomies in Table IV. The dry weights of samples of 100 eggs provided a sensitive quantitative assessment of the state of development of the ovary. From the graph, Fig. 58, it can be seen that egg dry weight in intact lampreys increases from approximately 7 mg. per 100 eggs in October, to 25 mg. per 100 eggs in April, most of the increase occurring after January. In every case, total hypophysectomy has prevented this increase in weight. The average weight of eggs from intact, control-operated lampreys examined during the same period did not significantly differ from the figure for normal lampreys at this time.

In oogenesis, unlike spermatogenesis, there is no evidence to suggest that egg development can still proceed, albeit at a slower rate, in the absence of the pituitary. Among the lampreys hypophysectomised in October, no.77 was killed on August 7th, nearly four months after the normal time of maturity. The dry weight of a sample of 100 eggs from this lamprey was 11.7 mg. This may be compared with 7.5 mg. and 11.1 mg. on May 10th (nos.73 and 74), 13.1 mg. on July 7th (no.75) and 13.8 mg. on April 26th (no.76). Similarly for lampreys hypophysectomised during later months, there was no indication that egg weight was greater in those animals which were allowed to survive longer (Table II).

TABLE III. Results of Hypophysectomy of female lampreys.

Date of operation	Lamprey No.	Date killed	State of sec. sex at death	Dry wt. of 100 eggs (mg.) At death		At operation
					monthly average	
Oct.19	73	May 10	None	7.5		
20	74	May 10	None	11.1		
20	75	Jul. 7	None	13.1	11.4	7.1
24	76	Apl.26	None	13.8		
25	77	Aug. 7	None	11.7		
Nov.14	78	Jun. 6	None	12.3		
14	79	Jun. 6	None	8.6		
14	80	Jun.13	None	16.5	14.2	8.1
14	81	May 8	None	9.9		
18	82	Apl.13	None	13.5		
18	83	Apl.15	None	18.1		
18	84	Apl.15	None	18.4		
21	85	Apl.15	None	16.6		
Dec. 5	86	Jun.13	None	16.1		
5	87	Apl.22	None	16.7		
5	88	May 8	None	14.4		
8	89	May 15	None	17.0	15.8	9.0
8	90	Oct.20	None	18.3		
88	91	May 8	None	13.2		
11	92	May 9	None	16.2		
13	93	May 15	None	14.7		
Jan.17	94	Aug. 7	None	18.3		
24	95	May 15	None	17.5	17.9	13.0
24	96	May 4	None	16.9		
24	97	Jun. 5	None	18.8		
Feb.19	98	Apl.13	None	19.3		
19	99	Apl.15	None	19.7	19.3	16.0
22	100	May 17	None	18.9		
Mar.13	101	May 15	None	18.6		
13	102	May 15	None	18.1	18.5	15.3*
17	103	Apl.17	None	18.8		

* Egg weight is lower than would be expected as lampreys in t group were smaller, having ascended the Severn in early spring (see p.56).

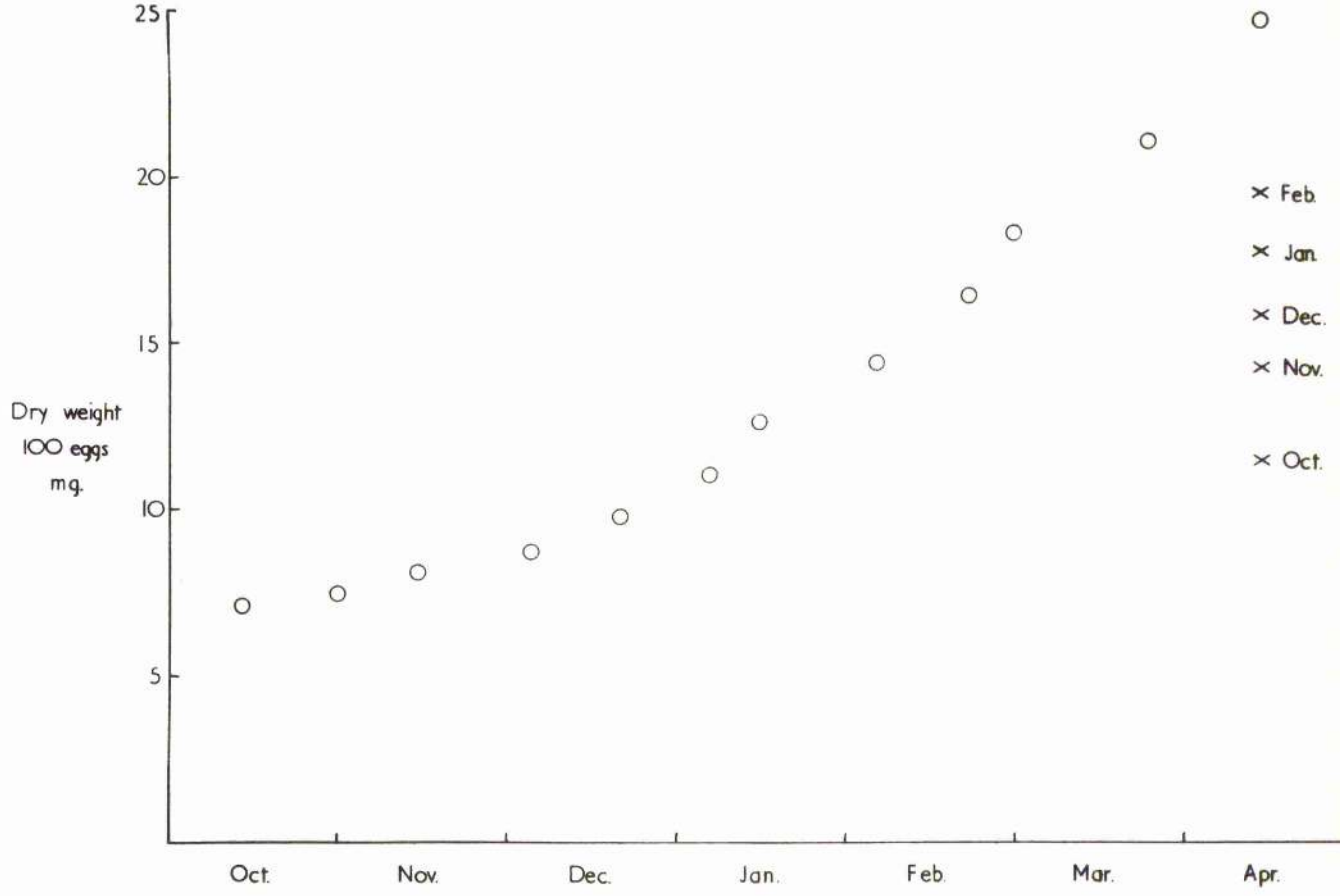
TABLE IV. Results of Control Hypophysectomy of female lampreys.

Date of operation	Lamprey No.	Date killed	State of sec. sex at death	Dry wt. of 100 eggs (mg.) At death		At operation
					monthly average	
Oct.20	104	Apl.13	Mature	24.6		
20	105	May 1	Mature	26.3	24.7	7.1
20	106	Apl.27	Mature	25.2		
25	107	Apl.27	Mature	22.8		
Nov. 6	108	Apl.20	Mature	27.0		
18	109	Apl.13	Mature	26.9	26.3	8.1
29	110	Apl.26	Mature	25.0		
Dec. 8	111	May 20	Mature	24.3	24.9	9.0
8	112	Apl.19	Mature	25.6		
Jan.17	113	May 6	Mature	24.8	25.6	13.0
17	114	Apl.20	Mature			
Feb.15	115	May 25	Mature	26.1	26.1	16.0
Mar.13	116	May 8	Mature	24.7	24.7	15.3

Fig.58. Graph showing dry weights of samples of 100 eggs taken from normal lampreys, and from lampreys hypophysectomised in the months shown. (see Table III).

o - Weights from normal lampreys.

x - Weights from hypophysectomised lampreys.


25
20
15
10
5
Dry weight
100 eggs
mg.
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
Feb.
Jan.
Dec.
Nov.
Oct.

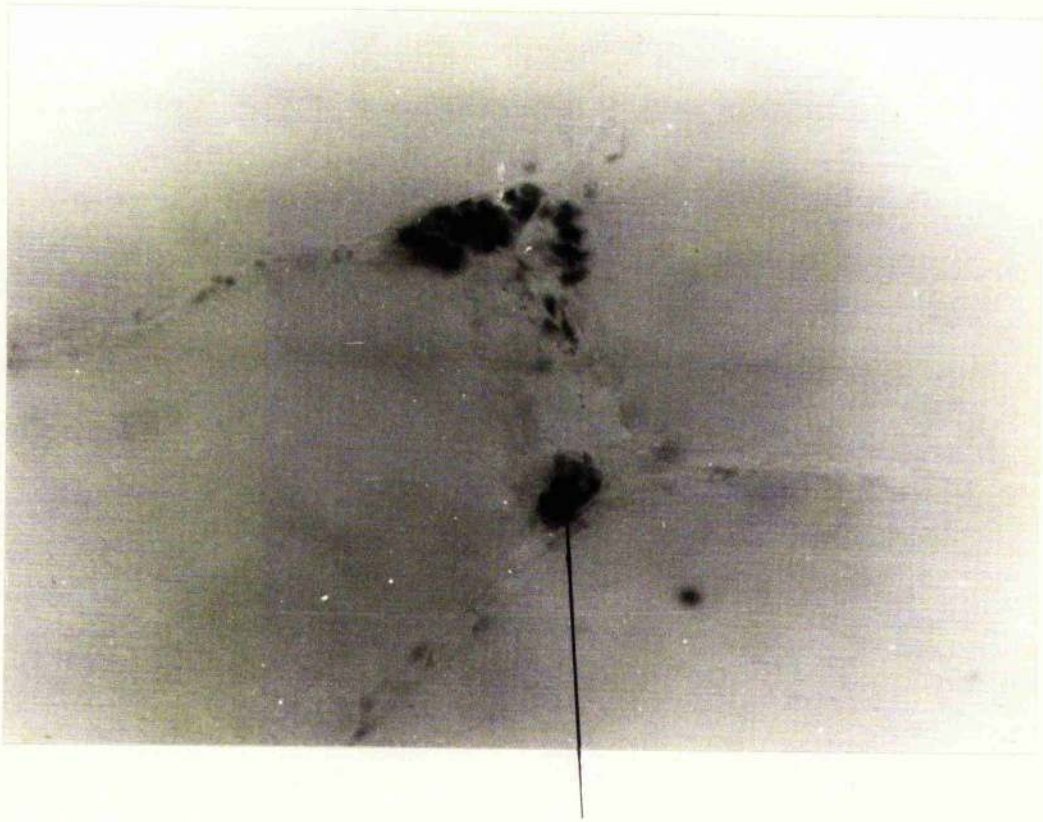
When the figures for the egg dry-weights of hypophysectomised lampreys in April to July are compared with the weights of eggs from normal lampreys at the time at which hypophysectomy was performed (Fig. 58 and Table IID), it can be seen that some small increase in egg weight appears to have occurred in the absence of the pituitary. This increase is fairly consistent for lampreys hypophysectomised between October and February, being between 3.3 and 6.5 mg. per 100 eggs, and does not appear to be dependent upon the weight of the eggs at the time of hypophysectomy. Taken as a percentag of the egg dry weights when the pituitary was removed, this increase naturally appears smaller in the larger eggs of the lampreys hypophysectomised in January and February, despite the probable greater capacity of the larger eggs for absorptio of food material due to their greater surface area. The increase in weight of the eggs after hypophysectomy thus appea to be more or less constant in lampreys hypophysectomised between October and February. Moreover, once this initial increase has taken place, egg weight appears to remain constan as shown above. The weights of the eggs from lampreys hypo-physectomised in March cannot be considered in this connection as the animals used for these experiments were from a differen stock, having ascended the Severn in early spring. The overa size of these lampreys was smaller than that of the autumn-run ones, and the eggs were smaller, though maturity was reached only slightly later than in the larger animals.

Apart from the differences in weight and size, the eggs from hypophysectomised lampreys differed from those of normal mature lampreys in several respects. On opening the body cavity of a hypophysectomised lamprey in April, or even as late as August or September, it was immediately apparent that ovulation had not taken place. The ovary remained as a compact body, the oocytes being bound together firmly by the connective tissue (Fig.59). In all respects the appearance was that of an immature ovary.

Histological examination of the ovaries from hypophysectomised lampreys confirmed the semblance of immaturity. In the eggs from lampreys hypophysectomised in October the cortical alveoli had decreased in size slightly, from 20-30μ to 15-20μ, and they occupied a peripheral layer in the egg approximately 100μ deep (Fig.60), as in the oocytes of normal lampreys examined in October. The cortical alveoli of lamprey hypophysectomised in November were similar in size to those of the October hypophysectomised lampreys and they occupied a zone of similar extent.

In normal lampreys during December and January, the cortical alveoli reduce in size and migrate peripherally to occupy a much narrower layer. Accordingly the ovaries of lampreys hypophysectomised during these months and later contained small, peripherally situated alveoli, this mature configuration having been attained before removal of the pituitary.

Fig.59. Comparison of ovaries of normal (above) and hypophysectomised female lampreys. Note the larger size of the eggs of the unoperated lamprey and the fact that they have been ovulated and are now loose within the body cavity. The eggs of the hypophysectomised lamprey (below) are small, and still bound together with connective tissue. (Natural size).



Interstitial cell

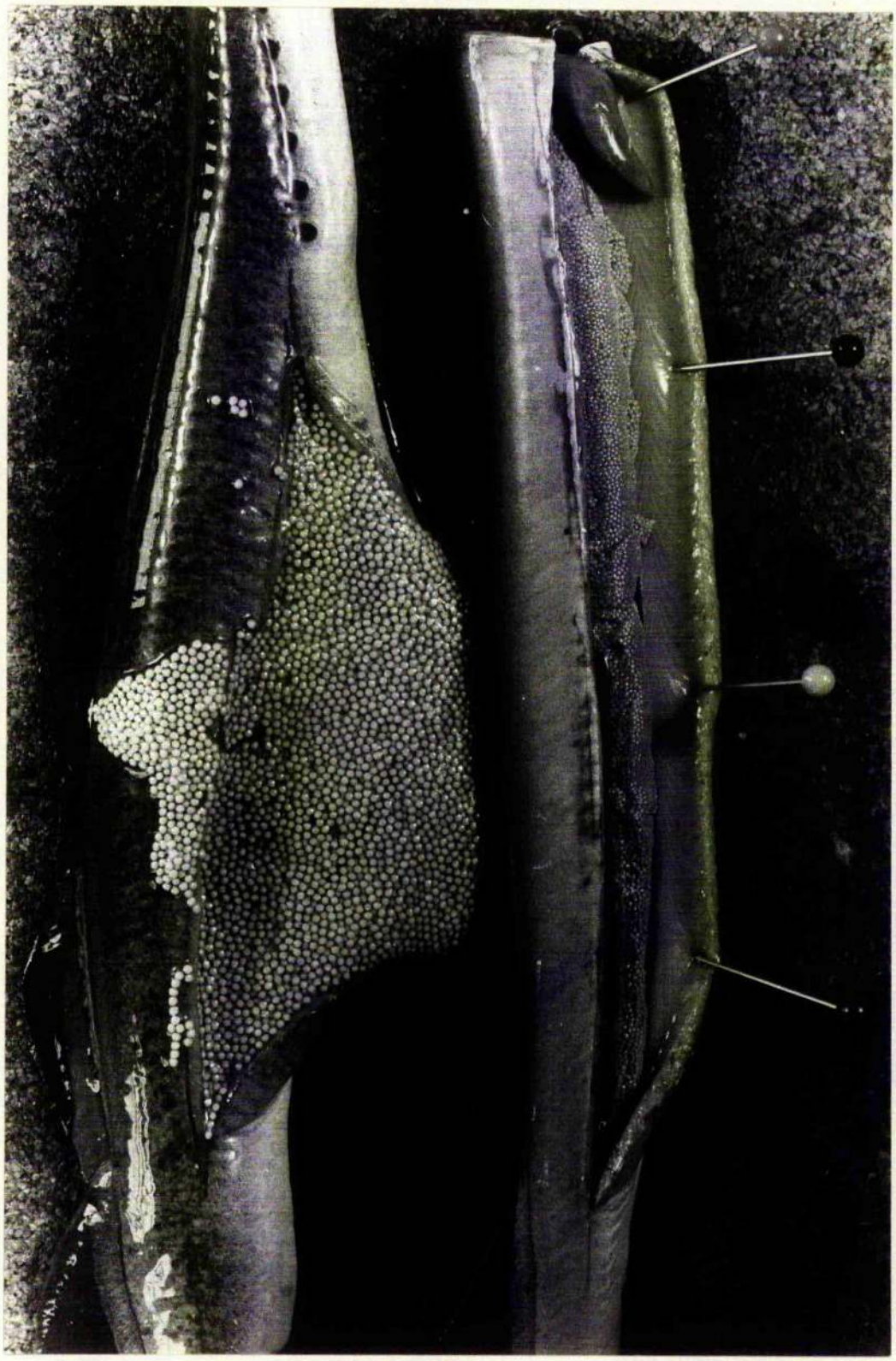


Fig.60. Vegetative pole of egg from lamprey no.75 hypophysectomised in October and examined in April, showing cortical alveoli still occupying a deeper band then in mature eggs (cf. Fig.45). (X 200).

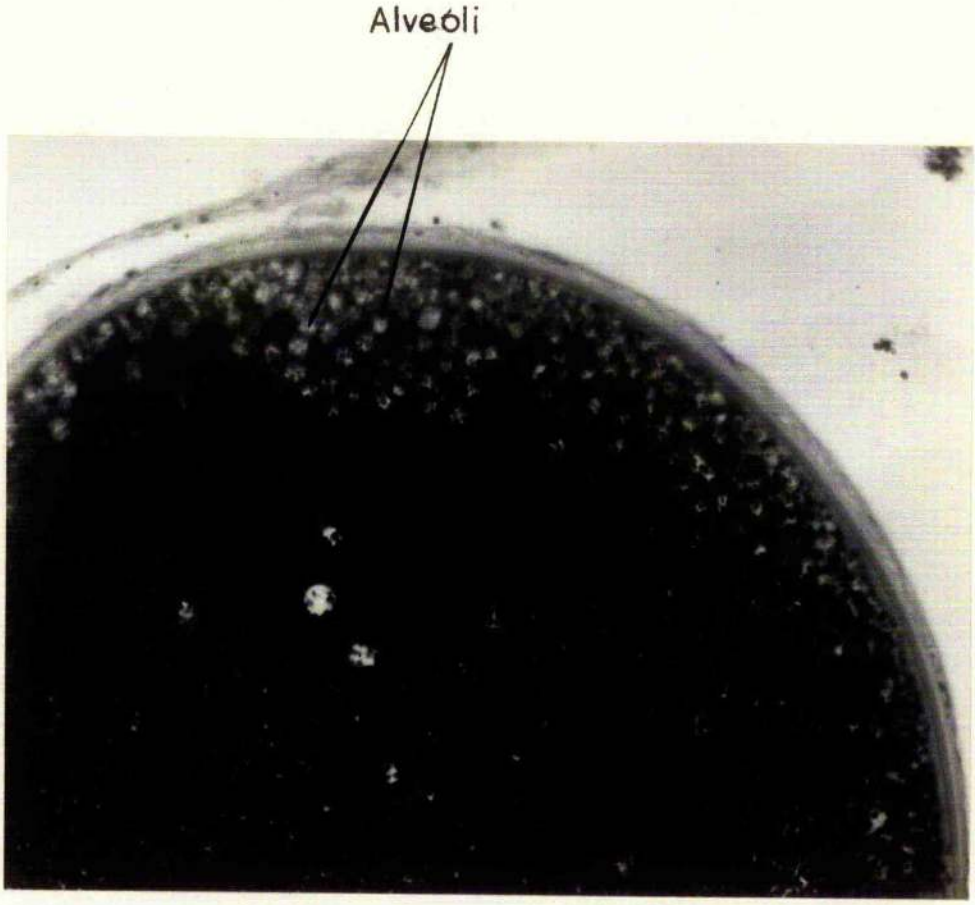
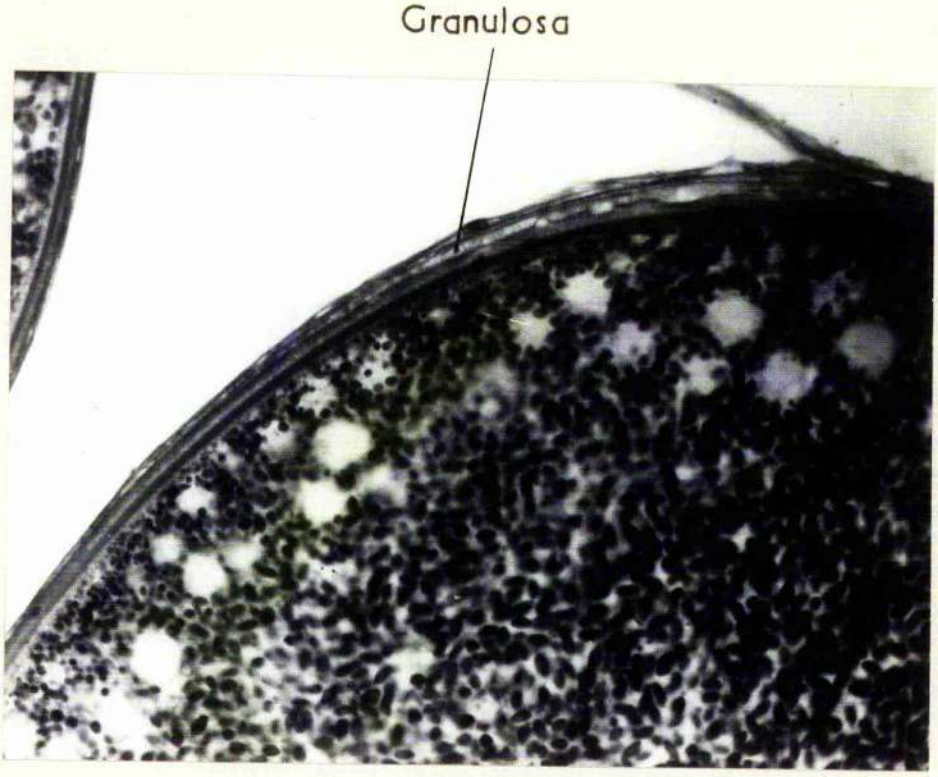
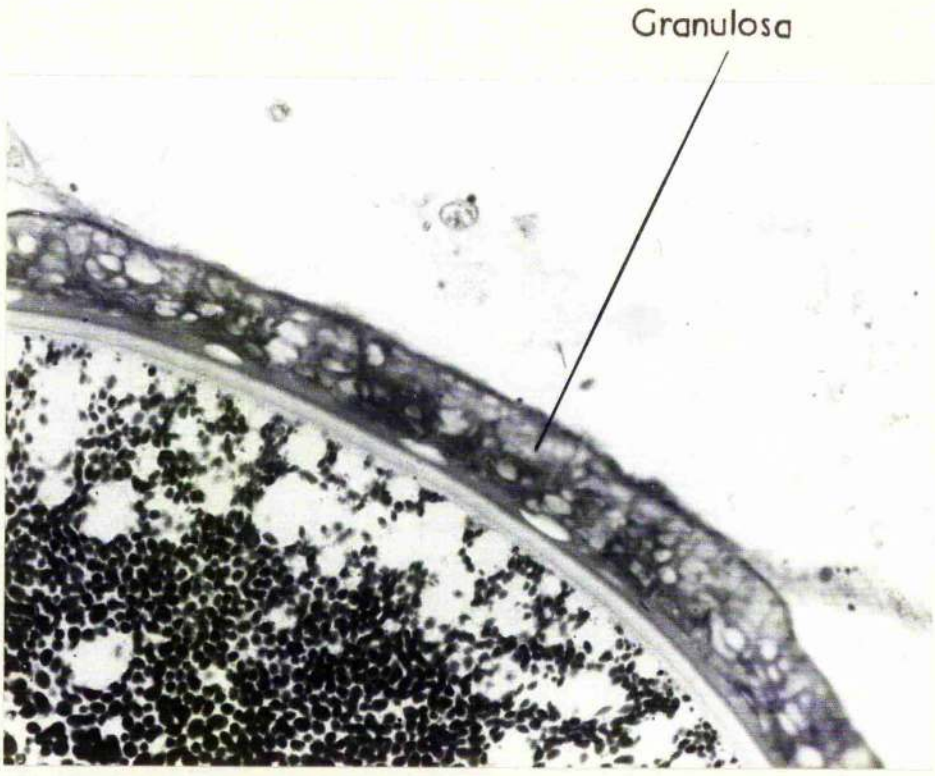

Alveoli

Fig.61. High-power view of follicle of egg from lamprey hypophysectomised in October, showing squamous granulosa (cf. Fig.62). (X 350).

Fig.62. Follicle of mature egg showing cuboidal cells of granulosa. (X 350).


Granulosa


Granulosa

Hypophysectomy caused no striking change in the appearance or thickness of the zona radiata or the theca. The most marked difference between the ovaries of hypophysectomised and normal lampreys, when examined in April or later was exhibited by the granulosa. In normal lampreys, during January, the granulosa begins to increase in thickness until by April it invests the vegetative half of the oocyte to a depth of 25-30μ. The general staining reaction of the granulosa in normal lampreys is strongly basophilic, though the cells also contain chromophobic inclusions. In April or later, lampreys hypophysectomised in October, November and December still had a very poorly developed granulosa, approximately 10-12μ thick (Fig.61). The granulosa of these anima resembled in appearance and thickness that of normal lampreys examined between October and December. The granulosa of lampreys hypophysectomised between January and March had developed to some extent when the pituitary was removed. Thu the depth of the granulosa in these animals was found to be geater than that of the earlier operated lampreys, ranging fro 15-20μ. During March the granulosa of normal lampreys is generally about 25μ thick (Fig.62). Lampreys nos.100, 101 and 102, hypophysectomised in March, were obtained from the spring run of smaller and less mature animals having a granulosa of 18μ in thickness. After hypophysectomy no increase in granulosa thickness occurred in these lampreys.

Hypophysectomy appeared to have no other effects on the ovary. The Sudan-positive, lipid-containing cells found in the theca of normal oocytes appeared to be unchanged after hypophysectomy, there being no significant increase or decrease in the amount of lipid present. Perhaps the most striking feature of the ovaries of hypophysectomised lampreys is the complete absence of follicles degenerating by atresia. As already stated, no such degenerating follicles occur in lampreys hypophysectomised at any time, or kept as much as four to six months after normal spawning time (more than nine months after hypophysectomy in the case of lampreys nos.77 and 90); the female lamprey is unique among vertebrates in this respect.

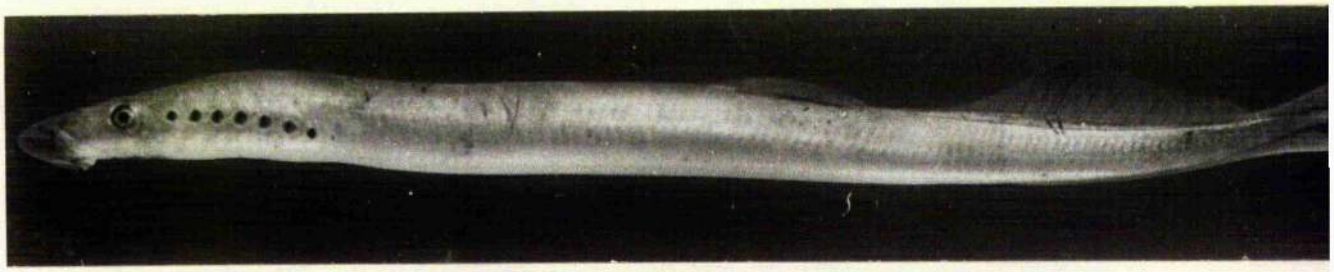
(iii) Effect on the secondary sexual characters.

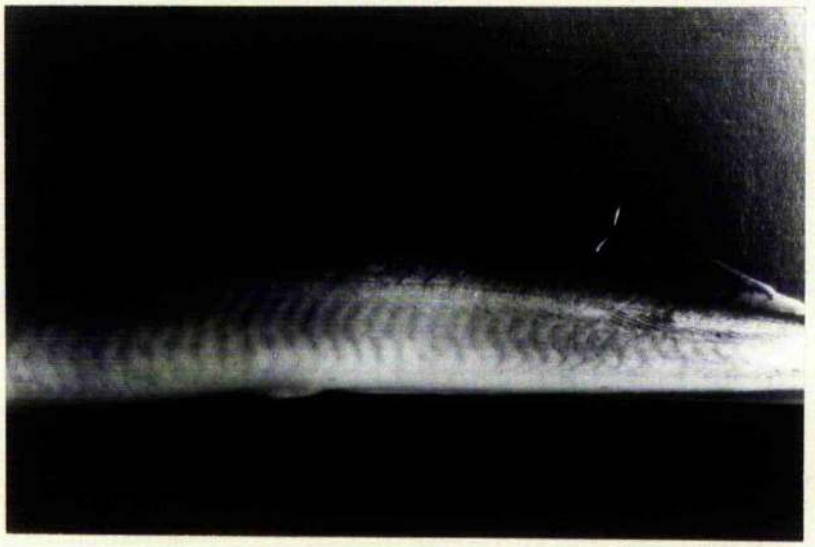
Total hypophysectomy of both male and female lampreys performed earlier than March resulted, in every case, in complete suppression of development of secondary sexual characters, the animals retaining the immature appearance in all respects (Figs. 63, 64). There was no sign of the normal enlargement of the posterior dorsal fin or of the swelling of the cloacal labia; the urinogenital papilla of the male lampre did not enlarge, and the female post-anal fin failed to develop In neither sex did the pores between the body cavity and the urinogenital sinus develop; thus hypophysectomised males containing sperm could not be 'stripped'.

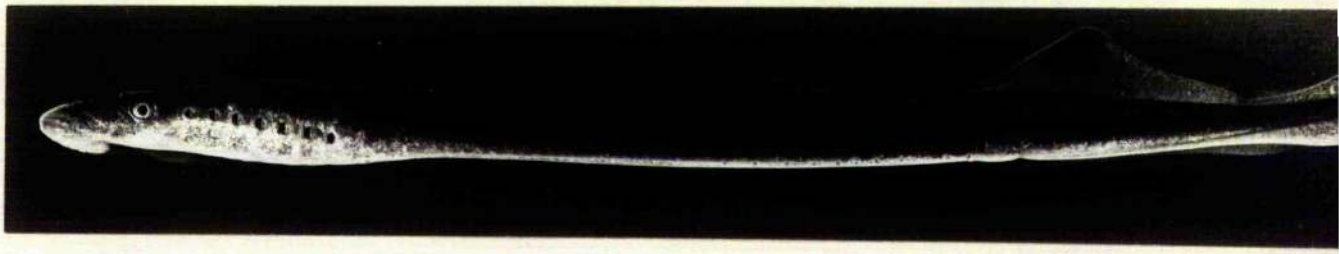
Fig.63. Hypophysectomised lamprey photographed in April. Note pallor, and complete absence of secondary sexual characters (cf. Figs.47 and 49).

Fig.64. Close-up view of cloaca of hypophysectomised lamprey showing absence of secondary sexual characters (cf. Figs.48 and 50).

Fig.65. Lamprey gonadectomised in November and photographed in April. No secondary sexual characters have developed (cf. Figs.47 and 49).







Secondary sexual character begin to develop in normal lampreys during March and lampreys hypophysectomised after this time might show slight secondary sexual characters at the time of operation. Animal no.54, recorded as displayi slight sexual characters at death, showed similar characters when it was hypophysectomised.

Hypophysectomised lampreys survive for a considerable time after the death of the stock of normal animals in April and early May. Many hypophysectomised lampreys survived in good condition into June, several into July and three (nos.55, 56 and 90) were still healthy and active when they were killed in September and October. Even in these long-lived specimens there was no sign of development of secondary sexual characters

2. GONADECTOMY.

Experimental results.

(i) Effects on secondary sexual characters.

Three lampreys (2 males, 1 female), gonadectomised in November, and seven (3 males, 4 females), in February, survived until April or May (see Table V). These animals were in good health and the long operation wound was completely healed at the time of autopsy.

None of the ten gonadectomised lampreys showed any sign of development of secondary sexual characters (Fig.65); the dorsal fins retained the immature appearance, the cloacal

labia did not swell, the urinogenital papilla of male lampreys failed to enlarge and the pores between the body cavity and th urinogenital sinus did not develop. The development of the secondary sexual characters in lampreys thus appears to be dependent upon a secretion of the gonad, as in other vertebrat

(ii) Effects on the pituitary.

The pituitaries of the ten gonadectomised lampreys were cut in serial section. The median section from each was chosen, stained with aldehyde fuchsin and the meso-adenohypoph photographed. The number of AF-positive cells in the meso-adenohypophysis was counted in a similar way to that employed for the normal lampreys (p.28). The results of these cell counts are shown in Table V , and in the graph (Fig.66).

The graph shows clearly that the number of AF-positi cells in the meso-adenohypophysis of gonadectomised lampreys i small (Fig.67, compare Fig.19), and similar to the number whic stain in early February in intact animals. It appears that the reinstatement of stainable material in these cells, which normally occurs during March and April has not taken place in the gonadectomised lampreys. The raised number of AF-positiv cells in lamprey no.120 is anomalous and no explanation suggest itself.

No differences were seen in the other regions of the pituitary, between normal and gonadectomised lampreys.

TABLE V. Results of Gonadectomy operations.

Lamprey No.	Date of operation	Date killed	Sex	Secondary sexual characters	No. of AF-positive cells in meso.
117	Nov. 6	Apl.16	F	None	10
118	Nov. 6	May 5	M	None	9
119	Nov. 6	May 6	M	None	11
120	Feb.15	Apl. 3	F	None	24
121	Feb.15	Apl. 4	M	None	11
122	Feb.15	Apl. 4	M	None	8
123	Feb.15	Apl.13	M	None	12
124	Feb.15	May 1	F	None	11
125	Feb.15	May 2	F	None	10
126	Feb.15	May 9	F	None	8

Fig.66. Graph showing the number of AF-positive cells in one section of the meso-adenohypophysis of normal lampreys (as Fig.20) together with the results of similar counts using gonadectomised lampreys. (see Table V).

o - Results from normal lampreys.

x - Results from gonadectomised lampreys.

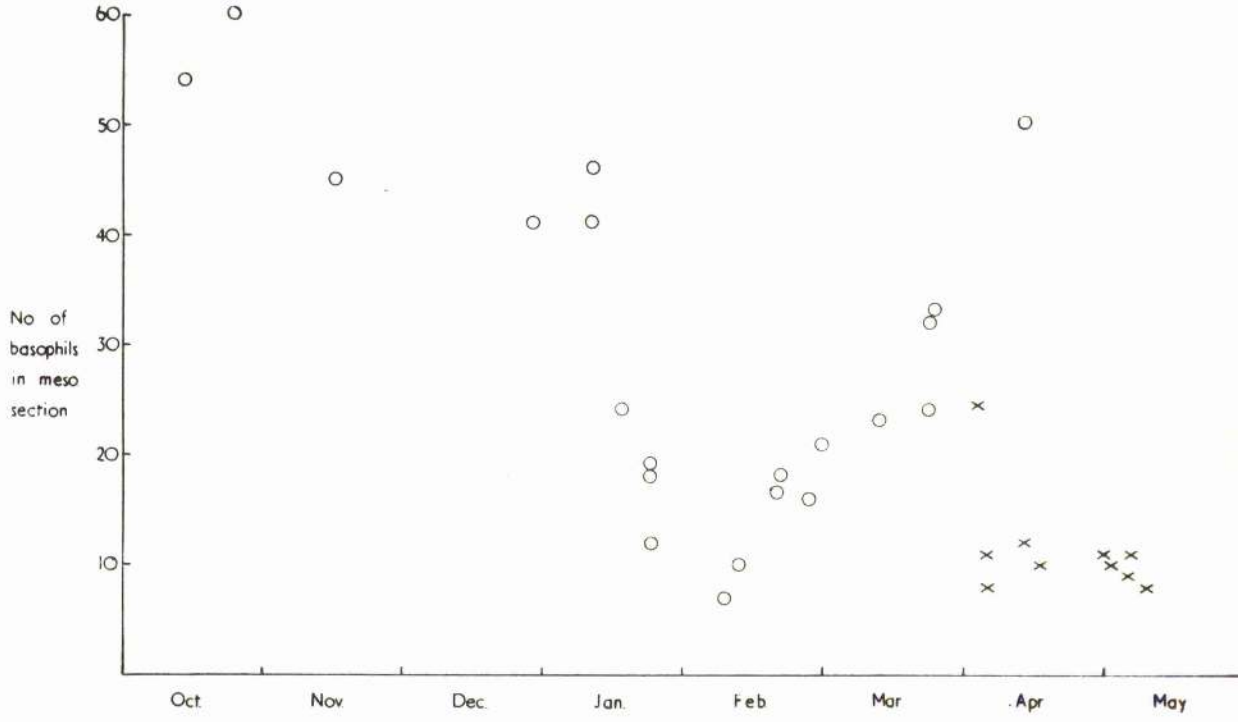
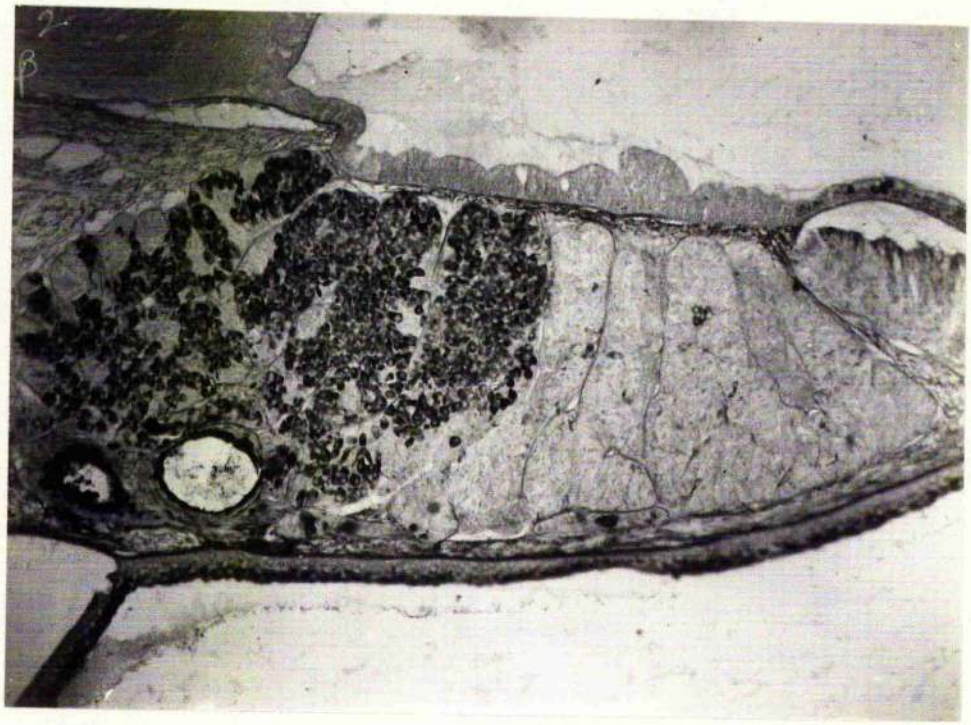

No of basophils in meso section
60
50
40
30
20
10
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May

Fig.67. Pro- and meso-adenohypophysis of gonadectomised lamprey, showing small number of AF-positive cells in the meso-adenohypophysis. (X 100).



3. INJECTIONS AND IMPLANTATIONS.

In any endocrinological investigation involving the surgical removal of a gland it is desirable to confirm that it is the absence of the organ which is in fact responsible for the post-operative changes. This can best be shown by the technique of 'replacement therapy' in which either extracts of the gland, or appropriate hormonal substances, are administered. Alleviation of the usual deficiency effects after such treatment provides confirmation that the removed gland has humoral control over the processes showing these effects.

In this study, the effects of pituitary removal were nullified by extracts of lamprey pituitary glands and by the mammalian gonadotrophins PMS and CG. Further, the stimulatory effects of the gonads on the secondary sexual characters were replaced by the administration of synthetic steroid hormones in hypophysectomised lampreys.

a) Review of literature.

There are several reports in the literature of attemp to accelerate development of the gonads and secondary sexual characters by treating lampreys with various hormone preparatio Calvet (1932) injected ammocoetes of Lampetra planeri with huma pregnancy urine. After two injections of 0.2 ml. urine, the ovary of the only surviving specimen was said to be more mature

than those of untreated animals. Calvet found survival to be better when the ammocoetes were bathed in one-third streng pregnancy urine for ten minutes on each of ten days. When the animals were killed, six days after the last treatment, the oocytes were seen to be greatly enlarged and the quantity of connective tissue in the ovary was reduced. There was no effect on the secondary sexual characters. Calvet concluded from these experiments that pregnancy urine contains an anterior pituitary-like gonadotrophic substance.

Damas (1933) injected adult L.fluviatilis with pregnancy urine during late February and March and found that the animals were stimulated to develop secondary sexual characters and fertile gametes approximately one month before the controls. Some stimulation of gametogenesis and seconda sexual characters was also obtained after injection with whol pituitary extract. The same author later carried out simila experiments (Damas, 1950) using lampreys on their spawning migration, the injections beginning in early December. Afte weekly injections of pregnancy urine, pregnancy uring extract or anterior lobe extract beginning in December and continuing into February, no stimulation of sexual development took plac compared with control animals. Damas carried out similar experiments at the same time, using testosterone propionate, also with negative results, and with considerable mortality.

The difference between the results of Damas' two experiments seems to be due primarily to the time of year at which they were carried out, since lampreys which have recent ascended the rivers, say in December as in the 1950 experimen may well be incapable of responding to hormone preparations which are effective when administered nearer the normal time for spawning. Dodd (1960) has suggested that experiments on the endocrine physiology of lampreys should be carried out we prior to the time of onset of sexual maturity. This would avoid any possible confusion of precocious sexual maturity with the effects of exogenous hormones, but unfortunately tak no account of the changing sensitivity of the tissues to gonadotrophin. It seems probable that the discordant result of the experiments described above are due to this difference in sensitivity, lampreys being incapable of responding early in the spawning migration to doses of hormone preparations whi are effective when administered later.

Young & Bellerby (1935) injected both larval and adult Lampetra planeri with cattle anterior pituitary extract between November and January. In the adults no gonadal changes occurred, but modifications of the cloaca and fins similar to normal secondary sexual characters took place. Si out of the seven lampreys treated died after between seven and thirty-one injections, suggesting that the dose (0.15 g. per injection) was rather large. The size of the dose could

account for the success in stimulating secondary sexual characters at a time when, from the experiments of Damas (195 it might be thought that sensitivity to gonadotrophins was lo The absence of any effect on gametogenesis suggests that the gametes at this time were still incapable of responding to stimulation by this dose of mammalian gonadotrophin. Simila injections of anterior lobe extract into ammocoetes also caused swelling of the cloacal labia, but with no sign of metamorphosis or gonadal development. Young describes 'abortive spermatogenesis' in three of the injected ammocoete and one adult. In these lampreys the chromatin of the germ cells was concentrated and, the cytoplasm broken up. It may be significant that a large number of the ammocoetes and adul died before the full course of injections had been administer and it is probable that the animals which survived were in poor condition. The degeneration of germ cells found in the experiments could well be a post mortem effect, or due to the poor condition of the animals.

Knowles (1939) injected extract of mammalian anteri lobe tissue into ammocoetes and adults of L.fluviatilis durin January and February. He found that the extracts caused no modifications of the fins, but that swelling of the cloacal labia occurred rapidly, often within twenty-four hours of injection, in both ammocoetes and adults. Knowles also described the appearance of the pores between the body cavity and the mesonephric ducts which occurs in normal lampreys

shortly before spawning. Injections of anterior lobe extrac caused the development of these pores in ammocoetes and adult

No gonadal changes were seen in the injected ammocoetes, but there was possibly a slight acceleration of spermatogenesis, two of the injected adults containing sperm on February 15th, approximately one month ahead of normal development. However, sperm was also found, on February 16t in a control adult injected with sesame oil, so the early maturation of the pituitary-treated lampreys might not have been a specific effect of the injections. Like Young & Bellerby, Knowles found evidence of degenerative changes in the testes of lampreys injected with pituitary preparations o with testosterone or oestrone, and in these experiments it is not suggested that the lampreys were moribund. After injections of testosterone or oestrone, the pores were found to develop between the body cavity and the kidney ducts of adults; these steroid hormones had no effect on ammocoetes.

Knowles suggested that the cloacal modifications of adult lampreys just prior to spawning might be a direct effec of pituitary gonadotrophins rather than an indirect effect vi the gonadal secretion of steroids, because of the rapidity of this response to injections, and the fact that steroids were found to have no effect on the secondary sexual characters of larvae, but cloacal modifications occurred after gonadotrophi treatment.

Lanzing (1959) briefly refers to his own experiments in which lampreys were injected with "at least 300 I.U. serum gonadotrophin or chorionic gonadotrophin between the latter half of February and the first half of March", presumably only one injection being given, though this is not specified. The lampreys developed secondary sexual characters about three weeks in advance of the controls. Extracts of lamprey pituitaries, about one gland per injection, also induced a premature appearance of the sexual characteristics, though again the number of injections is not stated. Lanzing makes no mention of the condition of the gonads of the injected lampreys.

When summarising the rather discordant results of the earlier investigators the effect of the time of year when the experiments were carried out must always be borne in mind, together with the acceleratory effect of the possibly higher temperatures under laboratory conditions. Thus the later experiments of Damas do not necessarily disprove his earlier findings; indeed, considering these early results (Damas, 1933) together with the work of Young & Bellerby, Knowles and Lanzing, it can be said that there is already some evidence for the stimulatory effect of mammalian pituitary extracts and pregnancy urine extracts on the sexual development of the lamprey.

b) Experimental results.

i) Injection of lamprey pituitary extracts into hypophysectomised lampreys.

Six hypophysectomised lampreys were injected with saline extracts of lamprey pituitaries at two to three week intervals between hypophysectomy and autopsy. The number of injections given to each lamprey, together with the results, are shown in Table VI. Each injection contained the extrac of three glands in 0.1 ml. 0.75% saline. Freshly removed pituitaries were used until the death of the stock of normal lampreys in early May. The last injection given to lampreys nos.127, 128 and 130 was of acetone-dried pituitaries collecte during April.

All six lampreys showed stimulation of the secondary sexual characters. Nos.127, 128 and 130, in which the degree of stimulation of these characters is described in the Table as 'fair', showed enlargement of the second dorsal fin and some swelling of the cloacal labia, though both to a lesser degree than the development of these characters in nos.129, 131 and 132, which were indistinguishable from normal mature lampreys. Development of secondary sexual characters in these hypophysec omised lampreys injected with lamprey pituitary extract took place several weeks later than in normal lampreys, indicating that a larger dose of extract would be required to simulate normal conditions more accurately.

Examination of the testes of lampreys nos.127 and 12 showed that spermatozoa were present, tightly whorled as in the normal mature testis. The spermatozoa of no.130 were slightly whorled, and no.128 showed randomly distributed sperm Had the animals been allowed to live longer, or if pituitary material had been injected in larger quantities or more frequently, it seems probable that the normal mature appearanc would have been attained by the whole group of lampreys.

The group of control lampreys, hypophysectomised and injected with 0.1 ml. saline at similar intervals to the experimental injections, showed characters similar to hypophysectomised lampreys receiving no injections. Nos.142, 143 and 144, hypophysectomised in December, showed no secondar sexual characters. The testis of no.142 when examined in April contained primary spermatocytes in diakinesis; nos.143 and 144, killed in May, contained randomly distributed sperm. The four lampreys hypophysectomised in February and injected with saline as controls showed very slight secondary sexual characters, the enlargement of the second dorsal fin having begun, and the cloacal labia being slightly swollen. These characters, however, were developed to this degree when hypophysectomy was carried out in late February, and did not develop further after the removal of the pituitary and injecti of saline. The sperm of nos.145 and 146 was slightly whorled but not more so than that of other lampreys hypophysectomised

127	M	19 Dec.	29 May	Pituitary	8	Sperm **	Fair
128	M	19 Dec.	17 May	Pituitary	8	Sperm	Fair
129	M	26 Feb.	27 Apr.	Pituitary	4	Sperm **	Mature
130	M	26 Feb.	21 May	Pituitary	5	Sperm *	Fair
131	F	26 Feb.	25 Apr.	Pituitary	4	Eggs loose	Mature
132	F	26 Feb.	29 Apr.	Pituitary	4	Eggs loose	Mature
133	M	18 Dec.	24 May	PMS.CG	8	Sperm **	Mature
134	M	18 Dec.	29 May	PMS.CG	8	Sperm **	Mature
135	M	18 Dec.	29 May	PMS.CG	8	Sperm **	Mature
136	M	18 Dec.	29 May	PMS.CG	8	Sperm **	Mature
137	M	18 Dec.	29 May	PMS.CG	8	Sperm **	Mature
138	M	26 Feb.	17 May	PMS.CG	5	Sperm **	Mature
139	M	26 Feb.	23 May	PMS.CG	5	Sperm **	Mature
140	F	26 Feb.	25 Apr.	PMS.CG	4	Eggs loose	Mature
141	F	26 Feb.	28 Apr.	PMS.CG	4	Eggs loose	Mature
142	M	18 Dec.	15 Apr.	Saline	7	Sc 1 Diak	None
143	M	18 Dec.	27 May	Saline	8	Sperm	None
144	M	18 Dec.	29 May	Saline	8	Sperm	None
145	M	26 Feb.	13 May	Saline	5	Sperm *	Very slight
146	M	26 Feb.	28 Apr.	Saline	4	Sperm *	Very slight
147	F	26 Feb.	22 Apr.	Saline	4	Eggs small, not loose	Very slight
148	F	26 Feb.	29 Apr.	Saline	4	Eggs small, not loose	Very slight

Sperm - Spermatozoa not whorled
Sperm * - Spermatozoa slightly whorled
Sperm ** - Spermatozoa more whorled, almost as in normal lamprey
Sc 1 Diak - Primary spermatocytes in diakinesis

in February, and receiving no injections.

Both female lampreys injected with lamprey pituitary extract (nos.131 and 132) had ovulated, the large eggs being loose within the body cavity. These animals, though hypophysectomised, were indistinguishable in all respetts from normal sexually mature lampreys. On the other hand, the control lampreys (nos.147 and 148), hypophysectomised and injected with saline, had compact ovaries containing eggs which had not ovulated, similar to those of uninjected hypophysectomi lampreys.

ii) Injection of mammalian gonadotrophins into hypophysectomised lampreys.

A group of nine lampreys, five hypophysectomised in December and four in February, received intramuscular injection of a mixture of Pregnant Mares' Serum (PMS) and Chorionic Gonadotrophin (CG) at intervals of between two and three weeks (Table VI). These two hormone preparations of extra-pituitary origin have predominantly follicle-stimulating and luteinising effects respectively. A mixture of the two preparations was used in these experiments because suitable lampreys were in short supply, and it was considered important to obtain a result. No evidence is available on the response of the lamprey to the separate administration of the two preparations. Each lamprey received 200 I.U. PMS and 300 I.U. CG dissolved in

0.1 ml. 0.75% saline at each injection. In every case the secondary sexual characters were stimulated, the lampreys hav a mature appearance in all respects, in contrast to the control lampreys receiving injections of saline alone (descri in i) above) which showed no development of secondary sexual characters after hypophysectomy. The secondary sexual characters of the group of lampreys receiving PMS-CG injectio developed at approximately the same time as those of normal lampreys.

All the hypophysectomised male lampreys injected with PMS and CG (nos.133-139) contained tightly whorled sperm when examined in May. While such whorling might possibly occur in a lamprey hypophysectomised in February and receivin no injection, its presence in lampreys hypophysectomised in December (nos.133, 134, 135, 136, 137) can be due only to the effect of the administered gonadotrophins.

The hypophysectomised female lampreys (nos.140 and 141) injected with PMS and CG, were killed in April, when the secondary sexual characters were fully developed. The ovari of these animals were mature, the eggs being large and loose within the body cavity. As described above, in control fema nos.147 and 148, hypophysectomised and receiving injections of saline, the ovary was compact and contained only small egg

Thus mammalian gonadotrophins of extra-pituitary origin appear to be capable of mimicking the gonadotrophic

activities of the pituitary gland in lampreys, these substanc stimulating normal development of both germ cells and seconda sexual characters.

iii) Injection of mammalian gonadotrophins into normal lampreys.

Fifteen normal lampreys (ten male and five female) were injected with a mixture of 200 I.U. PMS and 300 I.U. CG in 0.1 ml. 0.75% saline, receiving 14 injections between the start of the experiment on November 14th and its end on March 3rd; a similar group of control animals was injected with saline at similar intervals. The object of the experiment was to determine whether the injection of mammalian gonadotrophins could accelerate gamete production and the developme of secondary sexual characters. The experiment was ended and the animals killed in early March, at which time lampreys of the normal stock were beginning to show secondary sexual characters, and their testes contained late spermatids.

During February, when secondary sexual characters in normal and control lampreys had not begun to develop, the second dorsal fins of the PMS-CG injected lampreys enlarged and the cloacal labia showed signs of swelling. When the lampreys were killed in March, the secondary sexual characters of nine of the injected group were mature, while those of the remaining six, though less well developed, were still in advance of the characters of the control animals (see Table VI

Eight of the saline-injected lampreys showed no secondary sexual characters and in the remaining seven the second dorsal fins had undergone very slight enlargement. Sexual characters were developed to this extent in normal animals also, at that time.

The testes of all ten male lampreys injected with PMS and CG contained spermatozoa, and in five of these testes whorling of the sperm had begun. Sperm were not found in the testes of any of the control lampreys injected with saline. The testis of one saline-injected lamprey (no.164) contained spermatids alone and that of no.159 contained spermatids together with secondary spermatocytes. The testes of seven control-injected lampreys contained spermatids, secondary spermatocytes and late primary spermatocytes, and no.162 contained primary spermatocytes alone. The injection of the PMS and CG mixture had thus accelerated spermatogenesis by two to three weeks.

In the five female lampreys injected with PMS and CG, the effect of the hormone on the ovary was less marked than on the testis of the males. Determination of the dry weight of samples of 100 eggs from each of five PMS-CG injected and five saline-injected control lampreys showed no significant difference between the two groups, the weights being 17.6 mg. $\pm$ 2.37 for the gonadotrophin-treated lampreys, and 16.4 mg. $\pm$ 1.82 for the controls. Despite the lack of significance of

TABLE VII. Injection of mammalian gonadotrophins into normal lampreys.

Lamprey No.	Injection	Sex	State of Sec. sex	State of gonad	Dry wt. of 100 eggs	
149	PMS.CG	M	Slight	Sperm *		
150	PMS.CG	M	Slight	Sperm		
151	PMS.CG	M	Mature	Sperm *		
152	PMS.CG	M.	Mature	Sperm		
153	PMS.CG	M	Slight	Sperm *		
154	PMS.CG	M	Slight	Sperm		
155	PMS.CG	M	Mature	Sperm		
156	PMS.CG	M	Mature	Sperm		
157	PMS.CG	M	Mature	Sperm *		
158	PMS.CG	M	Mature	Sperm *		
159	Saline	M	Very slight	S'cytes 2 S'tids		
160	Saline	M	None	Sc 1 Diak S'cytes 2 S'tids		
161	Saline	M	None	Sc 1 Diak S'cytes 2 S'tids		
162	Saline	M	None	Sc 1 Diak		
163	Saline	M	Very slight	Sc 1 Diak S'cytes 2 S'tids		
164	Saline	M	None	S'tids		
165	Saline	M	None	Sc 1 Diak S'cytes 2 S'tids		
166	Saline	M	Very slight	Sc 1 Diak S'cytes 2 S'tids		
167	Saline	M	None	Sc 1 Diak S'cytes 2 S'tids		
168	Saline	M	None	Sc 1 Diak S'cytes 2 S'tids		
169	PMS.CG	F	Slight	Slightly friable	21.0	
170	PMS.CG	F	Slight	Slightly friable	17.5	
171	PMS.CG	F	Mature	Friable	14.8	Mean 17.6 mg. S.D. ±2.37
172	PMS.CG	F	Mature	Friable	15.3	
173	PMS.CG	F	Mature	Friable	19.4	
174	Saline	F	Very slight	Slightly friable	13.8	
175	Saline	F	Very slight	Slightly friable	18.3	
176	Saline	F	None	Firm	18.3	Mean 16.4 mg. S.D. ±1.82
177	Saline	F	Very slight	Firm	16.7	
178	Saline	F	Very slight	Slightly friable	14.8	

Sperm - Spermatozoa not whorled
Sperm * - Spermatozoa slightly whorled
S'cytes 2 - Secondary spermatocytes
S'tids - Spermatids
Sc 1 Diak - Primary spermatocytes in diakinesis

these weights, however, a certain difference was observed in the texture of the ovaries. In the normal female as ovulation approaches, the ovary becomes friable, being more easily broken up into individual oocytes. This presumably results from the weakening of the follicular membranes prior to their ultimate complete breakdown at ovulation. In the lampreys injected with PMS and CG the ovaries of three (which also showed mature secondary sexual characters) had become friable, and of the remaining two (with secondary sex characters less well developed) were deemed to be 'slightly friable'. Of the five saline-injected control lampreys, the ovaries in three were slightly friable, and in two they were quite firm.

iv) Effects of testosterone treatment.

Hypophysectomised and intact male lampreys, and three intact female lampreys were treated with testosterone in the form of pellets of approximately 12.5 mg. or 25 mg. implanted intraperitoneally, or with an aqueous suspension of testosteron propionate (Testaform, BDH) injected intramuscularly, as shown in Tables VIII, IX, X.

It was not possible to determine with accuracy the weight of testosterone absoebed from the pellets, due to the difficulty of recovering completely the portion of the pellet remaining unabsorbed at the end of the experiment. Over an experimental period of approximately five months, pellets of 25 mg. were reduced in weight by between 5 and 12 mg., and half

pellets of between 11 and 13 mg. were reduced by between 5 and 7 mg. It is probable that the smaller decreases in weight recorded, approximately 5 mg., give a more accurate estimate of the weight of testosterone absorbed by the lampre than do the larger decreases, which are undoubtedly due, to some extent, to fragmentation of the pellets.

Lampreys receiving intramuscular injections of the aqueous suspension of testosterone propionate were injected twice during the course of the experiments, with 2.5 mg. or 5 mg. of the steroid (2.5 mg. in 0.1 ml.), as shown in Tables VIII, IX.

In intact male lampreys (Table IX) implantation of pellets or intramuscular injections of testosterone had no effect on the development of the germ cells, all seven lampre containing whorled sperm of normal appearance when examined ir May. Development of the secondary sexual characters was not impaired, nor did these characters appear appreciably earlier in the testosterone-treated lampreys than in normal animals.

Twelve male lampreys hypophysectomised between Noven and February were treated with testosterone as shown in Table In the five lampreys which received implants (nos.179 to 183), the secondary sexual characters developed to the mature condit Lampreys nos.184, 185 and 186 each received two intramuscular injections of 2.5 mg. testosterone propionate, and the seconda sexual characters of these were judged to be moderately

TABLE VIII. Effect of testosterone or testosterone propionate on hypophysectomised male lampreys.

Lamprey No.	Date of Hypo.	Steroid given on	St. & type of steroid given	Form of steroid given	Date killed	State of sec. sex at death	Stage of germ cells at death
179	10 Nov.	10 Nov.	15 mg. T	Pellet IP	14 Jun.	Mature	Sperm **
180	10 Nov.	10 Nov.	14 mg. T	Pellet IP	22 May	Mature	Sperm **
181	10 Nov.	10 Nov.	12 mg. T	Pellet IP	30 May	Mature	Sperm **
182	10 Nov.	10 Nov.	14 mg. T	Pellet IP	21 May	Mature	Sperm **
183	29 Dec.	29 Dec.	25 mg. T	Pellet IP	4 Jun.	Mature	Sperm **
184	27 Jan.	27 Jan., 28 Feb.	2.5 mg. X2 TP	Inject. IM	26 May	Fair	Sperm *
185	27 Jan.	27 Jan., 28 Feb.	2.5 mg. X2 TP	Inject. IM	21 May	Fair	Sperm *
186	27 Jan.	27 Jan., 28 Feb.	2.5 mg. X2 TP	Inject. IM	3 Jun.	Fair	Sperm **
187	9 Feb.	9 Feb., 28 Feb.	5 mg. X2 TP	Inject. IM	25 Jun.	Mature	Sperm **
188	9 Feb.	9 Feb., 28 Feb.	5 mg. X2 TP	Inject. IM	13 Jun.	Mature	Sperm **
189	9 Feb.	9 Feb., 28 Feb.	5 mg. X2 TP	Inject. IM	4 Jul.	Mature	Sperm **
190	9 Feb.	9 Feb., 28 Feb.	5 mg. X2 TP	Inject. IM	30 Apr.	Mature	Sperm **

T - Testosterone

TP - Testosterone propionate

IP - Intraperitoneal

IM - Intramuscular

Sperm * - Spermatozoa slightly whorled

Sperm ** - Spermatozoa more whorled, almost as

TABLE IX. Effect of testosterone or testosterone propionate on intact male lampreys.

Fish No.	Steroid given on	Wt. & type of steroid given	Form of steroid given	Date killed	State of sec. sex at death	Stage of germ-cells at death
191	7 Nov.	12.8 mg. T	Pellet IP	26 May	Mature	Sperm **
192	7 Nov.	10.9 mg. T	Pellet IP	3 May	Mature	Sperm **
193	7 Nov.	11.5 mg. T	Pellet IP	22 May	Mature	Sperm **
194	7 Nov.	12.7 mg. T	Pellet IP	17 May	Mature	Sperm **
195	10 Jan.	25 mg. T	Pellet IP	29 May	Mature	Sperm **
196	10 Jan.	25 mg. T	Pellet IP	29 May	Mature	Sperm **
197	27 Jan., 28 Feb.	2.5 mg. TP	Inject. IM	22 May	Mature	Sperm **

T - Testosterone

TP - Testesterone propionate

IP - Intraperitoneal

IM - Intramuscular

Sperm ** - Spermatozoa tightly whorled, almost as in normal lamprey

developed during May and June. Lampreys nos.187 to 190 received two injections each of 5 mg. testosterone propionate, and the secondary sexual characters developed to maturity between April and June. Lampreys hypophysectomised at simila times and receiving no testosterone treatment developed no secondary sexual characters even when they survived as long as to June or July (Table I).

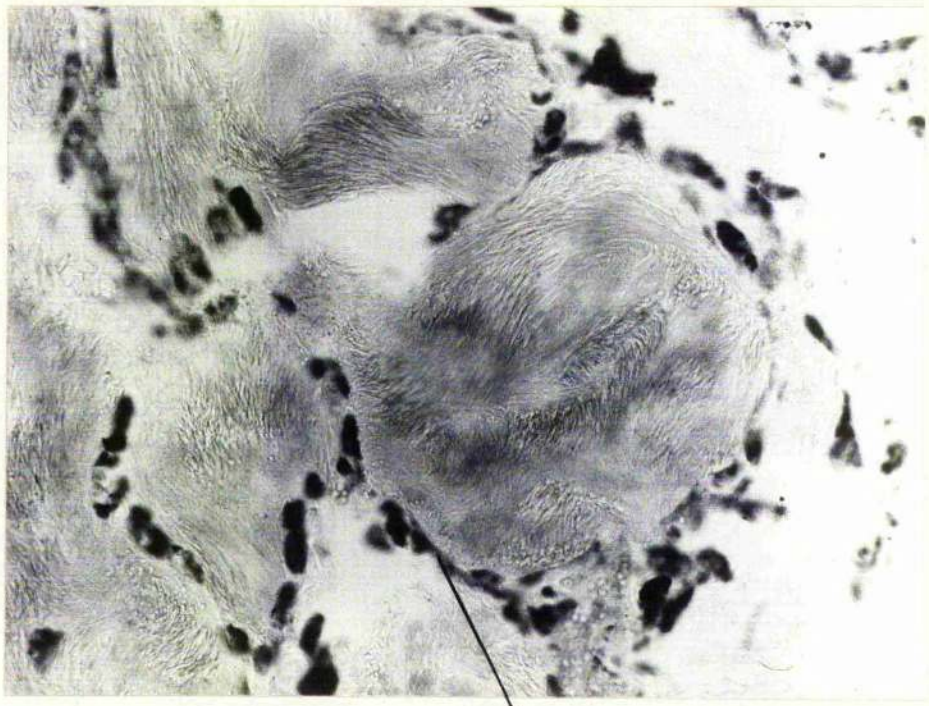
The testes of all twelve hypophysectomised male lampreys treated with testosterone contained sperm during May or June, either slightly whorled or completely whorled as in the mature testis. Hypophysectomised lampreys which received no testosterone treatment also contained, in some cases, sperm during May and June (Table I), though whorling had not occurred in any lamprey hypophysectomised in November or earlier, and only slight whorling was seen in a small number of December-hypophysectomised lampreys. It is unfortunate that no gonad biopsy samples were taken from the testosterone-treated lampreys during March or April when untreated hypo-physectomised lampreys rarely contain sperm, but intact lampre have mature gonads. Such samples would indicate whether testosterone treatment is capable of maintaining the progress of spermatogenesis at the normal rate (by the presence of sper or whether testosterone has no effect on spermatogenesis, whic proceeds at a slower rate in hypophysectomised lampreys. In the absence of evidence of the condition of the testes in Marc

and April this question cannot be resolved, though it may be significant that the degree of whorling of the sperm in lampreys nos.179 to 183 is unusual for lampreys hypophysectomised in December or earlier.

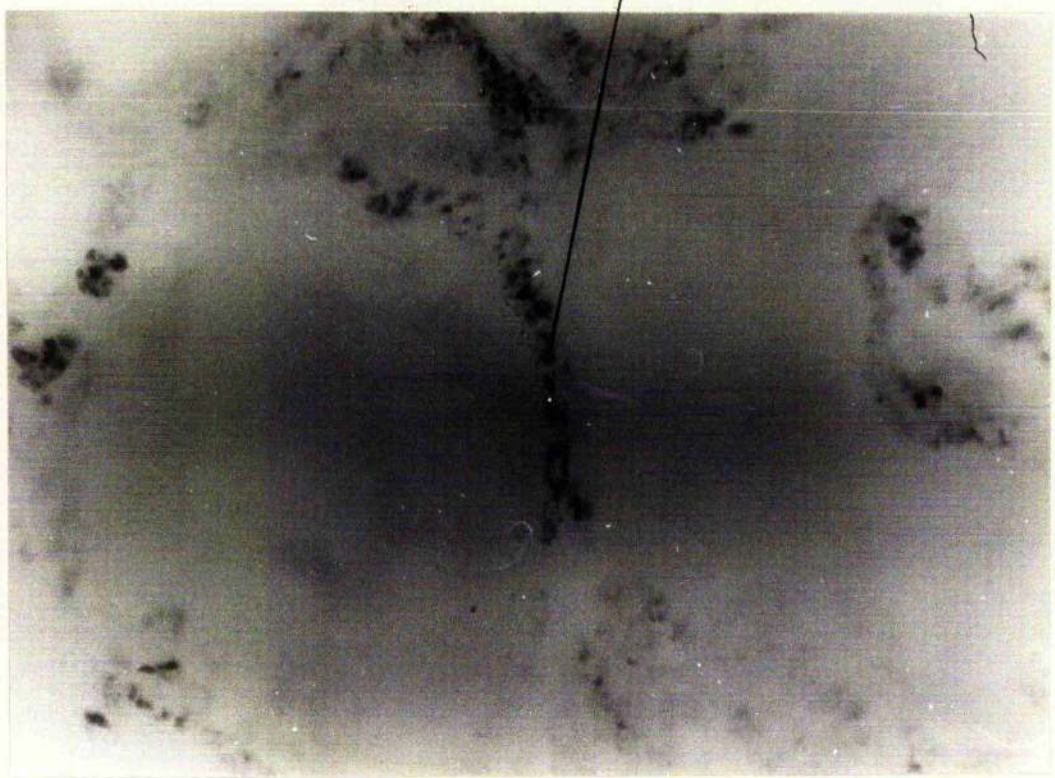
Treatment with testosterone appeared to have no effect on the Sudan staining of the 'interstitial' cells of the testes of intact or hypophysectomised lampreys, though distinct differences were seen in the lipids of the lobule walls. In intact and hypophysectomised lampreys receiving no steroid treatment and examined between April and June, the lipids of the lobule walls were in the form of small droplets. After testosterone treatment in both intact and hypophysectomised lampreys examined at sexual maturity, the lobule wall lipids were evenly distributed within the cells of the lobule walls (Fig.68). The Sudan staining of these lobule wal cells was less intense than that of the 'interstitial' cells. The Schultz technique demonstrated the presence of cholestero or its esters within these lobule wall cells, as well as with the 'interstitial' cells, in all the intact and hypophysectom male lampreys treated with testosterone (Fig.69). In normal mature lampreys no Schultz positive material could be demonstrated within the lobule walls (see p.37). This finding suggests that the cells of the lobule walls may be involved in the secretion of androgenic steroid hormones.

Fig.68. Sudan-stained section of testis from lamprey no.183 implanted with testosterone and hypophysectomised in December. Note the large accumulations of lipid within the lobule-boundary cells (cf. Fig.36). (X 250).

Fig.69. Positive Schultz reaction in both "interstitial" and lobule-boundary cells of lamprey treated with testosterone (cf. Fig.38). (X 250).



Lobule-boundary cells



Three female lampreys, two intact and one hypophysectomised, were implanted with testosterone pellets, as shown in Table X . When the animals were killed on May 22nd the secondary sexual characters of all three showed strong masculinisation (Fig. 71). In each animal the characteristically female post-anal fin was almost completely absent, and certainly not enlarged to a similar extent to the fin of a normal mature female. The second dorsal fin was enlarged, as occurs in mature lampreys of both sexes. The most striking effect of the testosterone was shown by the cloacal region of the lampreys. In the normal maturing female lamprey the cloacal labia become swollen, on the lateral and anterior borders of the cloaca, considerably altering the outline of the ventral body surface. This swelling completely obscures the small urinogenital papilla (Fig. 70). In a mature male lamprey a slight hyperaemia of the cloacal labia occurs, and the urinogenital papilla enlarges, protruding from the cloaca. In the three testosterone-treated female lampreys, the cloacal labia enlarged only slightly, as in the male, and the urinogenital papilla enlarged to more than 5 mm. in length: approaching the length of the papilla in a normal mature male lamprey (Fig. 71).

The dry weights of the eggs from these lampreys were rather higher than those of eggs from lampreys hypophysectomised during December and January, but still significantly below the

TABLE X. Effect of testosterone in female lampreys.

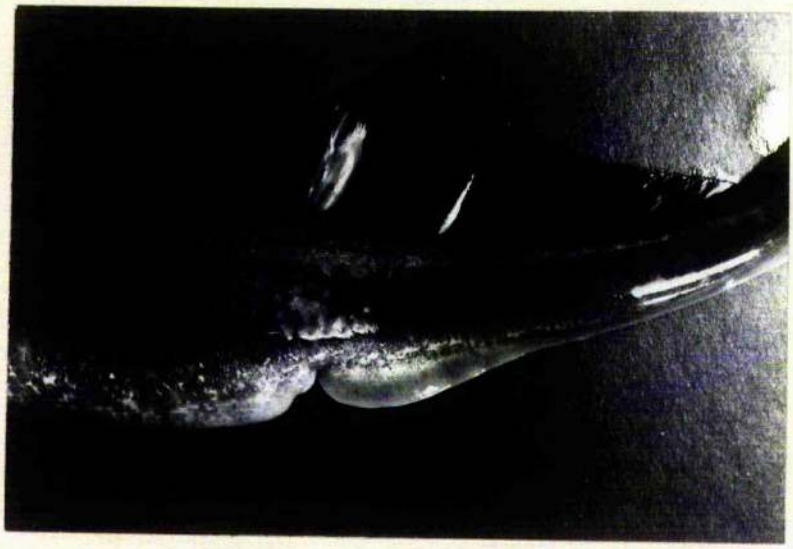
Fish No.	Intact or Hypo'd.	Testo-sterone given on	Wt. of steroid given	Form of steroid given	Date killed	State of sec. sex at death	Dry wt.(mg.) 100 eggs at death
198	Intact	29 Dec.	25 mg.	Pellet IP	22 May	Male *	20.1 mg.
199	Hypo.	29 Dec.	25 mg.	Pellet IP	22 May	Male *	17.9 mg.
200	Intact	10 Jan.	25 mg.	Pellet IP	22 May	Male *	19.9 mg.

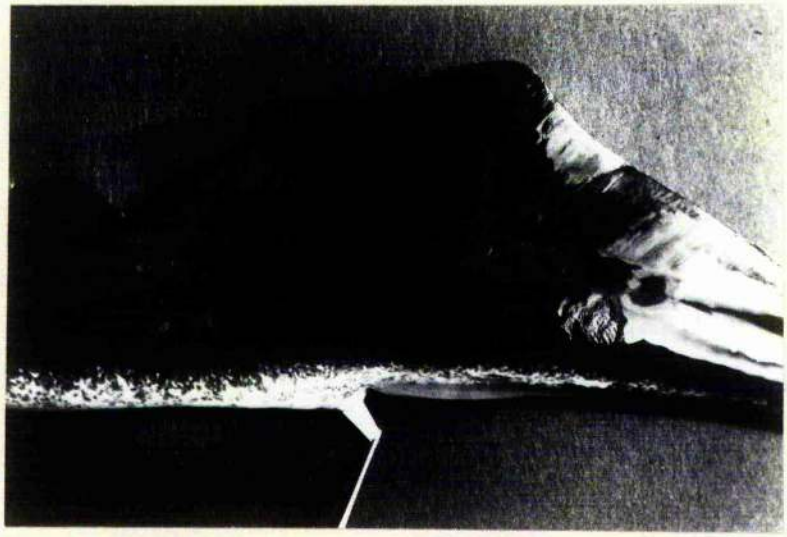
* = See text.

IP - Intraperitoneal.

Fig.70. Cloaca of normal female lamprey.

Fig.71. Cloaca of intact female lamprey implanted with testosterone in December and photographed in May. Note the absence of the characteristically female post-anal fin, and the development of the urinogenital papilla (held by forceps) similar to that of the male lamprey (Fig.48).





weights for mature lampreys (Fig.39). These eggs had the appearance of immature eggs when examined histologically.

4. IDENTIFICATION OF THE GONADOTROPHIC CELLS OF THE PITUITARY

Table XI shows the results obtained by the partial removal of the pituitary in ten lampreys. In each case serial sections were cut and the complete pituitary region examined in order to determine exactly the amount of tissue removed.

In assessing whether the gonadotrophic region of the pituitary had been removed, the development of secondary sexual characters was taken to be the most reliable criterion since even totally hypophysectomised male lampreys develop sperm.

In lampreys nos.201 to 207 the pro-adenohypophysis had been completely removed leaving the meso-adenohypophysis either intact (nos.201 to 206) or almost intact (no.207). In lampreys nos.201 to 205 both the meta-adenohypophysis and neurohypophysis were present in addition to the meso-adenohypophysis, and in nos.206 and 207 both these regions were absent. All these seven lampreys developed secondary sexual characters; in nos.206 and 207 the only pituitary tissue present was that of the meso-adenohypophysis.

Lamprey no.208 retained the meta-adenohypophysis and neurohypophysis intact, but both pro- and meso-adenohypophy were completely removed. This lamprey developed no secondary sexual characters.

Lampreys nos.209 and 210 contained some tissue from all four regions of the gland, but in no.209 the meso-adenohypophysis had been reduced to lateral remnants of small volume, the rest of the gland remaining intact, and in no.210 the volume of the pro-adenohypophysis had been considerably reduced. Lamprey no.209 developed only slight secondary sexual characters, while no.210 showed no effect of the operati

It is clear that when the meso-adenohypophysis is removed, development of secondary sexual characters is prevented. Reduction of the meso-adenohypophysis to small remnants (no.209) impairs, but does not prevent secondary sexual development. Conversely, removal of the pro-adenohypophysis alone, or together with meta-adenohypophysis and neurohypophysis has no effect on secondary sexual characters provided the meso-adenohypophysis is left intact. It can therefore be concluded that the meso-adenohypophysis is responsible for the secretion of the gonadotrophic hormone.

The data shown in the Table also confirm the finding of Young (1935) that the melanophore stimulating hormone is secreted by either the meta-adenohypophysis or the neurohypophysis. Lampreys nos.206 and 207 had contracted melanophores, and it was found that both the meta-adenohypophys and the neurohypophysis had been completely removed. Both these regions remained intact in the other lampreys shown in th Table, all of which had normally expanded melanophores. The

structure of the lamprey pituitary makes it difficult, if not impossible, to remove either the meta-adenohypophysis or the neurohypophysis alone, but it can safely be assumed that it is the meta-adenohypophysis which secretes the colour change hormone and is the homologue of the 'pars intermedia', and that the neurohypophysis of lampreys, like its homologue in higher vertebrates, serves as a storage centre for hormones formed in the neurosecretory centres of the brain.

TABLE XI. Results of partial hypophysectomy operations.

Lamprey No.	Secondary sexual characters	State of melanophores	Pituitary tissue remaining: Pro-adeno.	Meso-adeno.	Meta-adeno.	Neuro.
201	Mature	Expanded	-	+	+	+
202	Mature	Expanded	-	+	+	+
203	Mature	Expanded	-	+	+	+
204	Mature	Expanded	-	+	+	+
205	Mature	Expanded	-	+	+	+
206	Mature	Contracted	-	+	-	-
207	Mature	Contracted	-	Part	-	-
208	None	Expanded	-	-	+	+
209	Slight	Expanded	+	Remnants	+	+
210	Mature	Expanded	Remnants	+	+	+

DISCUSSION.

1. PITUITARY HISTOLOGY AND FUNCTION.

The histochemical examination of the pituitary gland was carried out in an attempt to localise the site of gonadotrophin secretion, by correlating the variations in staining of the cells of the gland with the probable level of gonadotrophin secretion.

No consistent changes were seen in the pro- or meta-adenohypophysis or in the neurohypophysis, and it seems probable that these regions are not involved in the secretion of gonadotrophic hormone. The changes in the meso-adenohypophysis, however, suggest that this region is responsible for gonadotrophin secretion.

It is not clear from the literature what criteria can be reliably employed to determine whether a pituitary cell is actively secreting or not, nor is it certain that criteria which apply to one kind of cell can necessarily be used in investigating other kinds of cell.

In the case of the 'basophils' (also PAS- and AF-positive) in the lamprey meso-adenohypophysis, minimal staining occurs in early February. At this time the secondary sexual characters, shown to be absolutely dependent upon the pituitary gonadotrophic secretion, are beginning to develop in both sexes. In female lampreys in early February the dry weight of the eggs

is still only half its value for mature eggs, and development of the granulosa has only just begun; egg growth and granulosa development are also processes entirely dependent on gonadotrophins. The male lamprey at any time shows less dependence on pituitary hormones for germ cell development than does the female, and it is not clear whether the first meiotic division and subsequent processes, which are occurrin in February, are in fact greatly affected by gonadotrophins.

However, minimal AF staining of the meso-adenohypoph cells does correspond closely to the onset of secondary sexua character development and ovarian development, and it is reasonable to suppose that the level of gonadotrophins in the blood would be high at this time. Thus the actively secreting pituitary gonadotrophs appear to lose their content of stainable material.

The reinstatement of staining which occurs during March and April is probably due to the presence of circulatin sex hormones from the gonads. Such hormones are known to exist in lampreys, from the evidence of the effects of gonadectomy and, indirectly, by the effect of testosterone on the secondary sexual characters. No source of steroid hormone has yet been located with certainty in the ovary, but in the testis the quantity of tissue which is potentially steroid-secreting increases during March. It is likely that reduced secretion of gonadotrophin occurs, due to a feed-back

action on the pituitary by the sex hormones. This view is supported by the evidence of the ten gonadectomised lampreys (including both sexes), where reinstatement of the staining did not occur during April or May. Removal of the source of sex hormone had prevented this feed-back from taking place

It cannot, however, be assumed that because a pituitary cell contains stainable material it is not secretin Indeed, there is evidence against this, for during October, November and December (see Fig. 20) the number of staining cells in a sagittal section of the meso-adenohypophysis is little short of maximal, yet it is during these months that the lamprey testis is most sensitive to hypophysectomy, and in the female the effect is greater than when the operation is performed later. It is possible that the affinity of these cells for this stain is a reflection of the balance between secretion and release of hormone; the stainable material possibly represents a reserve enabling the rate of release of secretion to temporarily exceed the rate of synthe On this hypothesis a cell secreting at a level within its rat of hormone synthesis would take the stain, and only at a time when maximal hormone release occurred would loss of staining take place; staining would reappear as soon as release of hormone from the gland returned to lower levels. These problems will only be resolved when it becomes possible to measure the level of circulating gonadotrophin throughout the year.

It is not, of course, proposed that all the cells of the meso-adenohypophysis are capable of gonadotrophin secretion. Most of these cells are probably genuine chromophobes; possibly as Lanzing (1959) suggests, some of them are functionally related to the acidophils of other vertebrate pituitaries. No acidophils were found in any region of the pituitary and this may be correlated with the fact that it is perhaps unlikely that secretion of growth hormone would be maintained long after the growth phase of the lamprey, which occurs in the sea before the beginning of the upstream migration. Apart from a very small number of chromophobes in the pro-adenohypophysis, the chromophobes of the meso-adenohypophysis are the only cells other than basophils in the 'anterior lobe' homologue (pro- and meso-adenohypophysis) which could represent the sources of growth hormone and adrenocorticotrophic hormone.

No evidence was obtained concerning the site of formation of the other pituitary hormones which, by analogy with other vertebrates might be expected to be present (Dodd et al., 1963). Dodd & Evennett (unpublished) have shown that a thyroid stimulatory substance is present in the pituita region of lamprey though there is no information on the identi of the thyrotrophs. van de Kamer & Schreurs (1959) believed that these cells lie in the meso-adenohypophysis in L.planeri (see p. 21 above for review) and, since they are AF- and PAS-

positive, it is possible that they are identical to the cells here considered to be gonadotrophs. van de Kamer & Schreurs hold that the gonadotrophic region of the pituitary is the pro-adenohypophysis. However, this now seems untenable in view of the evidence adduced above, nor is it likely that the thyrotrophs are located in this region. Dodd, Evennett & Goddard (1960) and Dent & Dodd (1961) have presented evidence that the thyrotrophic region in the dogfish Scyliorhinus caniculus is the ventral lobe and that this is probably the homologue of the meso-adenohypophysis. It may also be noted that in teleosts, thyrotrophic cells have been identified in the meso-adenohypophysis (Atz, 1953; Barrington & Matty, 1955) which "appears to contain all the cell types that are associate with the mammalian pars anterior" (Pickford & Atz, 1957). In contrast, in none of the vertebrates in which it is present has a function been assigned with certainty to the pro-adenohypophysis.

2. SECONDARY SEXUAL CHARACTERS.

The results of the experiments reported here indicate that in the lamprey, a representative of the most primitive group of vertebrates, the presence of the pituitary is essentia for normal development of the secondary sexual characters.

In lampreys, as in all other vertebrates which have secondary sexual characters, the development of these character

appears to be totally dependent upon the presence of a pituit gonadotrophin which causes the secretion of sex hormones from the gonads. This is evident from the fact that both hypophysectomy and gonadectomy in lampreys prevent the development of secondary sexual characters.

A mixture of the mammalian gonadotrophins of extrapituitary origin, PMS and CG, was found to be a completely effective replacement for the pituitary gonadotrophins, in inducing normal development of secondary sexual characters in hypophysectomised lampreys. This result presumably indicates that the mammalian gonadotrophins are capable of causing sex-hormone secretion by the lamprey gonad. It was not determine whether this effect was due to the follicle-stimulating or the luteinising component of the gonadotrophin preparation.

Evidence, additional to that provided by gonadectomy was obtained suggesting that the development of secondary sexual characters was due to the direct action of a gonadal hormone. Implantation of pellets of testosterone induced the development of male-type secondary sexual characters in both male and female hypophysectomised lampreys and in intact femal lampreys. The development of the male characters occurred in female lampreys with an intact ovary. This implies that the tissues of the female lamprey are capable of being masculinise by an androgenic steroid known to be secreted by the testes of higher vertebrates and that the nature of the secondary sexual

characters depends upon the kind of steroid administered, the secondary sexual tissues of the female lamprey at least being labile and capable of responding to both male and female hormones.

3. THE TESTIS.

a) Spermatogenesis.

Almost alone among vertebrates, the river lamprey spawns once only in its life history. In accord with this habit, the gonads produce only one crop of germ-cells. In the male, all the spermatogonia present in the testis undergo maturation to spermatozoa, and there is a high degree of synchrony of divisions between all the germ cells of an individual testis, and throughout the population. The course of spermatogenesis in the lamprey follows a similar path to that in other vertebrates.

It is generally agreed that secretions of the pituitary gland are essential for the normal development of the germ cells in vertebrates, although the results presented here indicate that the process of spermatogenesis in the lampre is unique in that it can proceed to completion from spermatogon albeit at a reduced rate, in the absence of the pituitary.

In teleost fish, several authorities are agreed that after hypophysectomy, spermatogonia retain their mitotic capaci but are unable to transform into primary spermatocytes (Matthew

(1939), Burger (1941) and Pickford (1953) working with Fundulus heteroclitus, and Vivien (1938, 1941) with Gobius paganellus). A similar effect of hypophysectomy was found in the elasmobranch Scyliorhinus caniculus by Dodd, Evennett & Goddard (1960), where 22 months after hypophysectomy the testis contained only spermatogonia and spermatozoa. Fish examined six weeks after hypophysectomy showed a zone of breakdown between spermatogonia and primary spermatocytes. It appeared that once spermatogonia had developed into primary spermatocytes, meiosis and maturation of the sperm continued unhindered.

Two different effectsoof hypophysectomy have been found by various authors working with Amphibia. Tuchmann-Duplessis (1945) using three species of Molge (= Triturus) found that hypophysectomy caused degeneration of spermatozoa and some secondary spermatogonia; primary spermatogonia remained intact. At the season when this work was carried out, no stages other than spermatogonia and spermatozoa were present in the testes. Walton (personal communication, 1962) working with Triturus cristatus found that hypophysectomy caused the formation of a zone of breakdown between spermatogo and primary spermatocytes. In newts kept for longer periods after hypophysectomy, degeneration of spermatocytes, spermatid and spermatozoa took place. Burgos (1949, 1950, 1955), using Bufo arenarum and Rana pipiens found degeneration of all stage of spermatogenesis.

On the other hand, Gallien (1938, 1940), van Oordt (1956, 1961) and Lofts (1961), all working with Rana temporari found that after hypophysectomy primary spermatogonia were unable to divide by mitosis to give rise to secondary spermatogonia, but "as soon as the secondary spermatogonia have developed into primary spermatocytes, spermatogenesis becomes independent of gonadotrophic hormone" (van Oordt, 1961

The effect of hypophysectomy on the testis of higher vertebrates is complex and the subject of some controversy. In general, however, it appears that after hypophysectomy spermatogonia fail to develop further, and that later stages of spermatogenesis degenerate. Thus there is a strong indication that in all groups of vertebrates previously studie the stage between spermatogonia and early primary spermatocyte or perhaps specifically the very early primary spermatocytes, is particularly vulnerable to the removal of gonadotrophic stimulation.

Spermatogenesis in the lamprey appears to be independent of pituitary gonadotrophins to a greater degree than in any other vertebrate. In teleosts, elasmobranchs and some amphibians, primary spermatocytes, once formed, can develop as far as mature sperm, but the formation of primary spermatocytes from spermatogonia in all these groups requires the presence of the pituitary. The results presented here indicate that in the lamprey after hypophysectomy even

spermatogonia retain the capacity to develop into mature sperm. There can be little doubt that the cells so described here are in fact spermatogonia since, during October, when many hypophysectomies were carried out, many metaphases were present in the testes. These can only be metaphases of spermatogonial mitoses, and could not be interpreted as meiotic metaphases since metaphase spindles of two distinct types are seen during February and March, these clearly representing the first and second meiotic metaphases. Moreover, the secondary spermatocyt stage in vertebrates is generally of short duration, the second meiotic division following the first division with little delay.

The experiments have not shown whether primary spermatogonia, preceding their last multiplicatory mitotic dividion, as well as the secondary spermatogonia formed by this division, are capable of development into spermatozoa in the absence of the pituitary. Since the final spermatogonial mitoses are occurring when the lampreys first become available in the autumn, this information is difficult to obtain with certainty. It is clear that some primary spermatogonia are present in the testes in October, for it is these cells that undergo the mitotic divisions seen in considerable numbers at this time. However, the possibility must remain, that these primary spermatogonia might have completed a hypothetical critical stage, perhaps in the prophase of their last mitosis, when the pituitary was removed in even the earliest hypophysect-

omised lampreys. It is probable that the mitoses seen in the testes in October are in fact the last mitoses of spermatogonia since by November the testes usually contain no metaphase

Hypophysectomy performed during October, November and December, when the germ cells were in the stages of spermatogonia and primary spermatocytes in early prophase, caused a delay of approximately two months in sperm formation. Hypophysectomy in January and later causes less delay, several lampreys hypophysectomised in this period containing whorled sperm in mid-April.

Hypophysectomy thus had its greatest effect on spermatogenesis when performed prior to the completion of the early part of the first meiotic prophase; removal of the pituitary during late prophase or later caused less delay in sperm formation. It is probable that the effect of the presen of pituitary gonadotrophins is exerted on a critical stage in the early course of the first meiotic prophase, though even this critical phase can be completed, at a reduced rate, in the absence of the pituitary.

While in the lamprey the pituitary appears merely to accelerate the first meiotic prophase, in higher animals hypophysectomy results in a complete block in spermatogenesis. In teleost and elasmobranch fish, and in the Amphibia, this block occurs between spermatogonia and primary spermatocytes at a point in spermatogenesis which corresponds closely to the

most sensitive stage in the lamprey. It is not known at which precise point in the transformation of spermatogonia to primary spermatocytes that degeneration occurs in the fish and Amphibia It can merely be said that degenerating cells occur, which are later in development than spermatogonia but earlier than the leptotene stage of primary spermatocytes.

The situation in the lamprey provides a useful clue to the understanding of the evolution of the pituitary's control over spermatogenesis in vertebrates. It seems reasonable to conclude that in the ancestors of the Cyclostomata, spermatogenesis was virtually independent of pituitary control. Lampreys, among the most primitive of living vertebrates, retain much of this autonomy, the pituitary merely accelerating the early part of the first meiotic prophase. In the fish and some Amphibia pituitary control over the earliest stage of the first meiotic prophase is established, while in other Amphibia, and reptiles, birds and mammals, gonadotrophins appear to be necessary for the maintenance of all stages of spermatogenesis later than spermatogonia.

While the above argument is in good agreement with the observed facts, several other interpretations must be borne in mind. The experiments have not shown whether spermatogonia which are not yet in the process of the last mitotic division also can develop to maturity in the absence of the pituitary. An absolute block in development between primary and secondary

spermatogonia would agree well with van Oordt's (1956) findings though in a later account van Oordt (1961) states that spermatogenesis becomes independent of gonadotrophic hormones "as soon as the secondary spermatogonia have developed into primary spermatocytes". This effect on the secondary spermatogonia is presumably in addition to the block in development between primary and secondary spermatogonia. The susceptibility of primary as well as secondary spermatogonial development to hypophysectomy appears to be a peculiarity of certain anuran Amphibia and possibly the elasmobranchs and is not paralleled in teleost fish where, in several cases, spermatogonial proliferation is known to occur after hypophysect (Matthews, 1939; Burger, 1941; Pickford, 1953). In the dogfish, Dodd (personal communication) has suggested that spermatogonial multiplication cannot take place in the absence of the pituitary since the testes of hypophysectomised fish contain only the normal number of spermatogonia. Had normal spermatogonial multiplication been able to continue, the number of spermatogonia would be expected to increase, as hypophysectom prevents the transformation of spermatogonia into primary spermatocytes (Dodd, Evennett & Goddard, 1960). However, in the absence of information to the contrary, it appears reasonabl to assume that in cyclostomes the mitotic divisions of spermatogonia are probably unaffected by hypophysectomy.

A second problem arises in cyclostomes concerning the question of whether the influence of pituitary gonadotrophi is exerted solely on the first meiotic prophase, or on the prophase and subsequent stages of spermatogenesis equally. The first meiotic prophase is longer by far than any other stage in spermatogenesis, the germ cells remaining in prophase for three months from mid-November to mid-February. By mid-March the testes generally contain spermatozoa, post-prophase stages of the first meiotic division, the second division and maturation to sperm taking place in one month. Thus even if the accelerating action of gonadotrophins were exerted on all stages equally the effect on the first meiotic prophase would appear to be greater. Sperm were present in April in the testes of lampreys hypophysectomised in late December, January and later, yet only one lamprey hypophysectomised before mid-December (no.7) contained even early sperm when examined in April. From these results it seems likely that a least the major part of the accelerating action of the pituitary on spermatogenesis occurs before mid-December, when the first meiotic prophase is in the leptotene stage. Without detailed results from a considerably larger number of animals, no definite conclusion can be arrived at on this point.

Hypophysectomy did appear to have an effect on the whorling of the sperm within the lobules, though this should not be considered as an effect on spermatogenesis or even perha

on spermateleosis. It is possible that the phenomenon of whorling is due not to the activity of the germ cells themselv but to the cells lining the lobules, and that the effect of hypophysectomy is indirect, due to deprivation of steroid hormones, rather than to the simple absence of pituitary hormones. This point of view is confirmed by the effect of testostefone on lampreys hypophysectomised during November and December (p.76), which showed almost normal whorling during May or June. No more than slight whorling was observed in lampreys hypophysectomised in December and receiving no steroid treatment, and no whorling was seen in untreated lampreys hypophysectomised in November.

b) Pycnotic nuclei.

In several hypophysectomised lampreys, and occasional in normal animals also, pycnotic nuclei were found in the teste The chromatin of these nuclei was tightly clumped and very densely staining. Similar nuclei were seen by Young & Bellerb (1935) and Knowles (1939) in lampreys treated with anterior lobe extract and testosterone respectively (see pp. 65, 66). Because of their occasional occurrence in normal animals and their rarity in the experimental animals, it was considered tha the presence of these pycnotic nuclei should not be interpreted as an effect of hypophysectomy. It appears likely that this degeneration of some germ-cell nuclei probably occurs after death, or in a moribund animal, and in several cases it was sho

that lampreys removed from the tanks after death contained such nuclei. The occurrence of few pycnotic nuclei in unoperated lampreys is probably due to the better condition of the intact animals, and to the fact that only freshly killed lampreys, fixed immediately after death, were used for the investigation of normal spermatogenesis.

Similar pycnotic nuclei are found in the testes of higher vertebrates after hypophysectomy, and are considered to be due to the removal of gonadotrophins, without which germ cells in certain stages of spermatogenesis cannot survive; if such were the case in the lamprey, pycnotic nuclei would be expected to occur more frequently. From the results of the hypophysectomy experiments it is clear that spermatogenesis in the lamprey can proceed even in the absence of the pituitary and that it is certainly not usual for a germ cell in any stage to fail to complete spermatogenesis.

c) Source of the male sex hormone.

In the testes of normal immature lampreys Sudan-positive substances (i.e. lipids) were found within cells which were interstitial in position, between the lobules. In appearance, these cells were very similar to the steroid-secreting Leydig cells of higher vertebrates; moreover, they reacted positively to the Schultz test for cholesterol and its esters.

In mature lampreys the Sudan and Schultz reactions of these 'interstitial' cells remained, and the cells forming the

boundaries of the lobules became Sudan-positive but not Schultz-positive. After treatment with testosterone these lobule boundary cells became Schultz-positive also.

In the pike, Lofts & Marshall (1957) have shown the presence of Schultz-positive material in a similar position in the lobule walls, and concluded that the lobule boundary cells represent the site of androgen production; the pike has no interstitial lipid-containing cells.

Until the results of testosterone treatment in lampreys were obtained, it was believed that the Sudan-positive material found in the lobule walls merely represented the products of fatty degeneration of the walls prior to their breakdown and the consequent release of sperm. Such an explanation appears reasonable, and may still represent the truth, but it is difficult to explain the occurrence of cholesterol in the lobule boundary cells of testosterone-treated lampreys without suggesting that these cells do in fact secrete a steroid hormone. If these cells were the source of the testicular hormone, the negative Schultz reaction in the mature lamprey would be explained by the supposition that the hormonal product was released as soon as it was made, insufficient being stored to react to the Schultz test. Testosterone treatment would be expected to reduce androgen secretion by the testis, by suppressing pituitary gonadotrophin production, and thus the hormone precursors might accumulate

within the lobule boundary cells. Two flaws in this argument suggest themselves. First, even if the lobule boundary cells in the normal mature lamprey were actively secreting, by analogy with other vertebrates a positive Schultz reaction should have been obtained, if only by virtue of the presence of hormone precursors, for example cholesterol itself. Secondly if, on the other hand, the lack of cholesterol in the mature lobule boundary cells were due to its immediate secretion, then a similar depletion of stainable material might be expected in the 'interstitial' cells also. The fact that the 'interstitial' cells and the lobule boundary cells react differently to the Schultz test in normal lampreys suggests that they do not share the same function. It would appear unusual for two different kinds of cell to secrete one hormone in the same animal, yet there is evidence for androgen production by the lobule boundary cells of the pike, and testosterone secretion by mammalian Leydig cells is undoubted.

It is obvious that the lobule walls of the lamprey testis must undergo some form of breakdown at maturity, in order to release the sperm into the body cavity; such degenerative processes are frequently accompanied by the accumulation of large quantities of lipid material (so-called 'fatty degeneration'). From the illustrations of Lofts & Marshall (1957), the mature testis of the pike is very similar

to that of the lamprey, with regard to the disintegration of the lobule walls and the release of the lobule contents. There is, however, an important difference between the testes of these two animals. In the lamprey, lobule wall lipid does not appear until spermatozoa are almost fully formed; in the pike Lofts & Marshall describe the presence of lipids within the walls more than six months before spawning, when many germ cells are still in the stages of spermatogonia and spermatocytes. The lipids in the pike can hardly be due to fatty degeneration of the lobules at such an early stage.

Thus while the situation in the pike appears to be similar to that in the lamprey, the resemblance may be superficial rather than functional, and it cannot be considered proved that the lobule boundary cells of the lamprey are the source of a steroid hormone. The effect of testosterone implantation on the lobule walls, causing them to give a positive cholesterol reaction certainly suggests a hormonal function for these cells, though it might be argued that cholesterol is a possible product of fatty degeneration, and that this degeneration is accelerated by androgens. From the evidence at present available it cannot be said with certainty which group of cells in the lamprey testis is responsible for steroid secretion.

4. THE OVARY.

In structure, the ovary of the lamprey is very similar to the typical non-mammalian vertebrate ovary, except that all the oocytes are always at the same stage of development since the lamprey spawns only once. Each oocyte contains a large quantity of yolk, and the follicle wall consists of the granulosa covering the vegetative hemisphere and the theca, which invests the whole oocyte.

Seen in histological section the granulosa remains squamous and almost chromophobic until the January preceding spawning, when it increases in size rapidly and strongly basophilic material appears in the cytoplasm. It has been suggested (Kille, 1960) that the granulosa cells give rise to the sticky jelly-like substance which covers the vegetative half of the ovulated eggs. This jelly fastens the eggs to stones in the bed of the river in the optimum position for the entry of spermatozoa.

So far the site of production of the female gonadal hormone has not been identified with certainty. From the results of gonadectomy experiments there is no doubt that the ovary secretes a substance necessary for the development of secondary sexual characters. By analogy with higher vertebrates and the male lamprey, the female gonadal hormone of lampreys might be expected to be a steroid, giving positive Sudan and Schultz reactions.

Apart from the densely Sudan-staining material contained by the yolk, very little lipid was found in sections of ovary. The only region other than the yolk which showed any Sudan staining was the theca, and the staining here was not sufficiently intense to provide unequivocal evidence of the presence of lipids. No positive Schultz reaction was obtained in the theca, though a faint positive could easily have been masked by the non-specific yellow-brown colouration produced by the action of the reagents on the tissues. In higher vertebrates it is thought that the theca is responsible for oestrogen secretion; in the lamprey the most likely region of the ovary for hormone secretion also appears to be the theca.

The experiments have shown that hypophysectomy of the female lamprey results in inhibition of vitellogenesis and prevention of granulosa development. Both these effects might be expected, from a knowledge of the effects of hypophysectomy in higher vertebrates, but the lamprey appears to be unique among vertebrates in the complete absence of atresia after hypophysectomy, as well as in the normal lamprey. Atretic follicles have been found in intact animals from all groups of vertebrates, including the cyclostome Myxine glutinosa (Lyngnes, 1936), and their absence from the lamprey ovary is thus unusual. Even in lampreys hypophysectomised for nine months (no.77, October 25th-August 7th; no.90, December 8th-October 20th) there was no sign of degeneration

of the oocytes. In every case after hypophysectomy, ovarian development ceased and the condition of the gonad did not change throughout the whole post-operative period. This long survival beyond the normal spawning time of the lampreys (4 or 6 months) is probably the maximum obtainable, and should it be that atresia in the lamprey is an extremely slow process then it will probably never be possible to demonstrate it since hypophysectomy much earlier than September of October, and survival beyond the following October would be difficult to achieve.

However, it is probably not reasonable to expect atresia after hypophysectomy in the lamprey, when no similar process occurs in the intact animal. In other vertebrates which have several breeding cycles in their lives, atresia is a normal process occurring after ovulation. Unovulated oocytes which have developed beyond a certain stage are caused to degenerate, only young oocytes remaining for development prior to the next spawning. It is probable that this atresia is due to a reduction in the level of circulating pituitary gonadotrophins, and thus a similar process might be expected after hypophysectomy. The lamprey, on the other hand, spawns only once in its lifetime, and apparently has not developed the mechanism for atretic degeneration of the oocytes. In the normal lamprey no advantage would be gained from atresia and in fact all the oocytes within the ovary are

ovulated prior to spawning.

Here it is possible to show a similarity between the male and the female lamprey. Spermatogenesis in the male lamprey appears to be capable of continuing, and all cells remain in good condition, after hypophysectomy. In higher animals a greater dependence on pituitary hormones has evolved, gonadotrophins being obligatory for the normal progress of spermatogenesis and even for the maintenance of the germ cells; after hypophysectomy in certain vertebrates, degeneration of some stages of spermatogensis ensues. In the female lamprey no atretic degeneration occurs after hypophysectomy. It is suggested that, just as the male germ cells of higher vertebrates have become dependent upon pituitary hormones for their existence, a similar dependence has developed in the female. The presence of degenerating oocytes within the ovary of an animal with several breeding seasons would be expected to favour the development of an organised activity of the follicle, concerned with removing the products of degeneration and reclaiming the food materials deposited in the yolk. This degenerative and resorptive process, found in representatives of all groups of vertebrates but not in the lamprey, is known as atresia.

From the evolutionary point of view therefore, the pituitary's control of gametogenesis in the lamprey is of great interest. The results obtained from the male show

that spermatogenesis in a vertebrate can proceed independentl of pituitary control, though the beginnings of the pituitary' effect are evident in the stimulatory effect of gonadotrophin In all higher groups of vertebrates the presence of pituitary hormones is essential. The dependence of oogenesis on gonadotrophins in the lamprey is greater; but while the process cannot proceed in the absence of the hormones, gonadotrophins are not essential for the maintenance of the germ cells. Even among cyclostomes, the ovary of Myxine contains atretic follicles (Myxine has several breeding seasons), and one might reasonably speculate that an effect of hypophysectomy in this animal would be the production of many corpora atretica. In the teleosts and all the higher vertebrates development and maintenance of the oocytes are dependent on the presence of gonadotrophins, and in all these groups atretic degeneration of oocytes is found.

5. REPLACEMENT THERAPY.

In hypophysectomised lampreys, normal gonadal and secondary sexual character development was maintained by injections of whole lamprey pituitary extract or a mixture of PMS and CG. The results of the injection of pituitary extracts confirm that the pituitary is in fact the site of gonadotrophic hormone production. It was not considered possible to fractionate the glands for differential injection

of, say, the pro-adenohypophysis or the meso-adenohypophysis, but sufficient evidence for the gonadotrophic functions' residing in the latter is provided by the surgical and histological results (pp.

The mixture of PMS and CG also provided an effective replacement for the pituitary in hypophysectomised lampreys demonstrating the effectiveness of mammalian gonadotrophic hormones in even the most primitive vertebrate group. Moreover these two gonadotrophic hormones, found in the serum of pregnant mares and in human pregnancy urine respectively, originate not in the pituitary gland but in the endometrium and the chorion respectively. This suggests that the gonadotrophin molecules have probably not materially altered in the course of several hundred million years of evolution. Separate administration of PMS and CG was not carried out, so no information is available on the distinctive effects of FSH and LH.

Intact lampreys were also injected with the PMS-CG mixture from mid-November, and in these animals secondary sexual characters became visible during February and the gonads approached the mature condition at the beginning of March, about one month ahead of the untreated control lampreys. Gonadotrophic hormones had thus been circulating at or above the normal level for three months before any effect on secondary sexual characters was noted. This suggests that

the tissues involved in the development of secondary sexual characters do not become sensitive to gonadotrophins until between one and two months befofe the normal spawning time; the tissues concerned are either those of the cloaca and fins, or the androgen-secreting tissue of the testis. Similarly it appears that gametogenesis is either incapable of proceedin at a very much faster rate even in the presence of abnormally high levels of gonadotrophic hormone, or is not fully sensitive to the effect of the hormone until a certain time or a certain stage has been reached. It is possible that temperature is the factor controlling the sensitivity of both the secondary sexual characters and gametogenesis to gonadotrophins. There is no doubt that a considerable rise in temperature occurs, both in the rivers and in the laborator tanks, in early spring at a time which might correspond well with the beginning of development of the secondary sexual characters. No detailed results are available, but there is evidence that lampreys mature earlier when kept in warmer conditions (Damas, 1950). A similar effect of temperature on gametogenesis has been noted in teleosts by Nusenbaum (1950) and by Spaul & Bullough (1942) and in Amphibia by, among others, Galgano & Falchetti (1940) and Ifft (1942).

It is extremely likely that the effects of temperatu and tissue sensitivity were responsible for the failure of Damas (1950) to repeat his earlier results. In the first

paper, Damas (1933) reported a striking stimulation of the gonads and secondary sexual characters by pregnancy urine and anterior lobe extracts injected during late February and March. On repeating the experiments earlier in the migrator period, beginning in December, Damas (1950) obtained no sexua stimulation.

With the two discordant results of Damas thus explained, the work reported here agrees with the work of previous investigators (Calvet, 1932; Damas, 1933, 1950; Young & Bellerby, 1935; Knowles, 1939; Lanzing, 1959), and shows that mammalian gonadotrophic hormones provide an effective substitute for the gonadotrophic substance of the lamprey's pituitary.

Implantation of testosterone into intact lampreys in January did not cause the secondary sexual characters to develop appreciably earlier than in control lampreys. This evidence supports the theory of low sensitivity of the secondary sexual characters to androgens until early spring, though it must be borne in mind that the presence of exogenou steroids probably depressed the normal steroid secretion of the testis (by reduction of gonadotrophic secretion), and tha the level of circulating hormone might have been little above the normal value in these animals.

In hypophysectomised lampreys, testosterone or testosterone propionate proved to be a suitable replacement

for the pituitary-controlled secretion of the testis. Steroid treated lampreys developed secondary sexual character and their sperm became whorled, the degree of whorling being considerably greater than that found in untreated lampreys hypophysectomised at similar times (see p.76).

It cannot be supposed that testosterone is necessarily the precise hormone produced by the lamprey testis, or even possibly by the mammalian testis, but treatment with this steroid caused normal development of secondary sexual characters in hypophysectomised lampreys. From their experiments, Young & Bellerby (1935) and Knowles (1939) could not be certain that secondary sexual characters in lampreys were not due to a direct action of pituitary gonadotrophins. In the experiments reported here, gonadectomised lampreys with a functional, even hyper-secreting pituitary gland, fail to develop secondary sexual characters, as did hypophysectomi lampreys with a normal gonad. Therefore there now seems little reason to doubt that secondary sexual characters in the lamprey are due to the secretion of steroid hormones by the gonads, evoked by gonadotrophic stimulation from the pituitary.

In conclusion, it can be said that reproduction in the lamprey is under the control of the pituitary gland,

and the nature of this control appears very similar to that in higher vertebrates. It seems that pituitary hormones influence both gametogenesis and the development of secondary sexual characters, the latter indirectly, by way of the gonadal hormones.

The gonads and secondary sexual characters responded to preparations of mammalian hormones, suggesting that little change has occurred in the nature of the hormones during several hundred million years of evolution. Concerning reproductive endocrinology, if not all of endocrinology, evolution appears to have been extremely conservative.

SUMMARY.

1. The thesis records an investigation into the endocrine control of reproduction in the lamprey.

THE PITUITARY GLAND.

2. The pituitary gland of the lamprey is described, and the results of staining histological sections of the gland with the periodic acid-Schiff and aldehyde fuchsin techniques are presented. The connection between the neurosecretory centres of the brain and the neurohypophysis is illustrated.

3. A variation with time in the number of cells within the meso-adenohypophysis reacting to aldehyde fuchsin is described, and correlated with the state of sexual development of the lampreys. It is shown that the number of aldehyde fuchsin-positive cells in the meso-adenohypophysis is minimal at the time when development of secondary sexual characters begin, and it is concluded that this is due to the release of stainable material, the hormone, from the cells at this time of maximal sexual development.

4. Histological sections of the pituitary glands of gonadectomised lampreys are described, and counts of aldehyde fuchsin-positive cells in the meso-adenohypophysis presented. It is shown that most of the cells of the meso-adenohypophysis lose their affinity for the stain after gonadectomy, and it is suggested that this denotes that the cells are hyper-secret and contain no stored hormone. These changes in the meso-

adenohypophysis are the only consistent changes in the pituitary after gonadectomy.

THE TESTIS.

5. The literature concerning spermatogenesis in the lamprey is reviewed and normal spermatogenesis described and illustrated.

6. Lipids are shown to be present in cells situated interstitially in the testis throughout the whole of the spawning migration, and in the cells of the lobule walls of the testes of mature lampreys. The Schultz reaction for cholesterol was found to give a positive result for the 'interstitial' cells but not for the lobule boundary cells in both immature and mature normal lampreys.

7. The results show that hypophysectomy retards the progress of spermatogenesis in male lampreys by up to two months, but that in the absence of the pituitary spermatozoa can ultimately develop even from germ cells at the spermatogonial stage. It is shown that in lampreys hypophysectomised later in the pre-spawning period, when development of the germ cells is more advanced, less retardation of spermatogenesis is caused.

8. The 'whorling' of mature sperm within the lobules of the normal testes is described, and it is shown that this whorling does not occur to its fullest extent in hypophysectomised lampreys.

9, The distribution of lipids and Schultz-positive material in the lamprey testis was found to be unchanged after hypophysectomy.

10. The effects of hypophysectomy in the lamprey are discussed and compared with the findings for other lower vertebrates. It is suggested that in the lamprey the action of the gonadotrophic hormones is limited to accelerating part of the prophase of the first meiotic division.

THE OVARY.

11. The literature on the lamprey ovary is cited and the normal development of the oocyte preceding spawning described. Data are presented illustrating the increase in dry-weight of the oocytes throughout the season. The absenc of atretic follicles and corpora lutea from the lamprey ovary is commented upon.

12. The presence of lipids in the theca but not in the granulosa of the ovarian follicle is shown. No positive result was obtained by applying the Schultz test to sections of ovary.

13. Hypophysectomy was found to prevent the normal increase in dry-weight of the oocytes in female lampreys. Development of the follicular granulosa was also prevented by hypophysectomy, and histological sections of oocytes reta the immature appearance in all respects.

14. The absence of atretic follicles from the ovaries of hypophysectomised and normal lampreys is discussed.

THE SECONDARY SEXUAL CHARACTERS.

15. The secondary sexual characters of male and female lampreys are described.

16. Hypophysectomy was found to suppress the developmen of secondary sexual characters in both sexes.

17. Sham hypophysectomy operations in which the pituitary is left intact had no effect on the secondary sexual characters or gametogenesis.

18. The results of partial removal of the pituitary gla in ten lampreys are presented. It is shown that whenever th meso-adenohypophysis is left intact, even in the absence of other regions of the gland, normal sexual development occurs; in the absence of the meso-adenohypophysis, development of secondary sexual characters does not take place, and gameto-genesis is impaired. From this result and from those quoted above (nos.3 and 4) it is concluded that the meso-adenohypoph is the source of the gonadotrophic hormone.

GONADECTOMY.

19. The results of gonadectomising ten lampreys are presented. Gonadectomy was found to prevent the development of secondary sexual characters, and it is thus concluded that a gonadal secretion is responsible for the initiation of thes characters in normal lampreys.

REPLACEMENT THERAPY.

20. The literature describing the effects of exogenous hormone preparations on lampreys is reviewed.

21. Lamprey pituitary extract was found to support norma development of secondary sexual characters and 'whorling' of sperm in hypophysectomised lampreys.

22. Similarly, a mixture of the mammalian hormones PMS and CG provided an effective replacement for the pituitary in hypophysectomised lampreys. PMS and CG accelerated gameto-genesis and development of secondary sexual characters when injected into normal lampreys.

23. Implantation or injection of testosterone caused the development of secondary sexual characters in hypophysect omised male lampreys.

24. Lipids, staining by Sudan black, were found in the 'interstitial' cells and lobule boundary cells of testosteron treated intact male lampreys, as in the normal mature testis. Schultz-positive material indicating the presence of choleste was found in these lobule boundary cells; the possibility that these cells might be responsible for the secretion of the androgenic hormone is discussed.

25. Intact and hypophysectomised female lampreys receiving implants of testosterone developed secondary sexual characters of the male type, and egg-growth was inhibited.

APPENDIX.

Periodic acid-Schiff technique.

Schiff's reagent (Coleman, 1938).

Dissolve 1 g. of basic fuchsin in 200 ml. boiling distilled water. Cool to 50°C and filter. To the filtrate add 2 g. potassium metabisulphite and 10 ml. N hydrochloric acid. Allow to bleach in the dark for 24 hours. Shake with 0.5 g. activated charcoal for 1 minute. Filter.

Method of staining (after McManus, 1948).

1. Bring sections to water.
2. Oxidise for 2 minutes in 0.5% aqueous periodic acid.
3. Wash in distilled water.
4. Treat with Schiff's reagent for 15 minutes.
5. Wash in running water 5-10 minutes.
6. Dehydrate and mount.

Aldehyde fuchsin techniques.

A. After Gomori, 1950.

Preparation of stain.

Add 1 ml. concentrated hydrochloric acid and 1 ml. paraldehyde to 100 ml. 0.5% basic fuchsin in 70% alcohol. Keep at room temperature until it becomes deep violet (approximately 4 days).

Method of staining.

1. Bring sections to water.
2. Oxidise for 2 minutes in:

5% sulphuric acid	10 ml.
2.5% potassium permanganate	10 ml.
distilled water	60 ml.

3. Bleach in 2% potassium metabisulphite.
4. Stain 2 minutes.
5. Differentiate in 1% hydrochloric acid in 70% alcohol.
6. Dehydrate and mount.

B. After Gabe, 1953.

Preparation of stain.

Stock solution: Dissolve 1 g. basic fuchsin in 200 ml. boiling water. Boil for 1 minute. Cool and filter. To the filtrate add 2 ml. concentrated hydrochloric acid and 2 ml. paraldehyde. Follow reaction by spotting on a filter paper, filtering when red has disappeared (usually after 4 days at 20°C). Dry the filter paper and precipitate to eliminate hydrochloric acid and paraldehyde. Dissolve to saturation in 70% alcohol.

Staining solution:

Stock solution	25 ml.
70% alcohol	75 ml.
Glacial acetic acid	1 ml.

Method of staining.

1. Bring sections to water.
2. Oxidise for 2 minutes in:

5% sulphuric acid	10 ml.
2.5% potassium permanganate	10 ml.
distilled water	60 ml.

3. Bleach in 2% potassium metabisulphite.
4. Stain 15 minutes.
5. Differentiate briefly in 1% hydrochloric acid in 70% alcohol.
6. Dehydrate and mount.

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